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COMPARATIVE STUDY OF THE HISTO-PHYSICS OF THE AORTA[1]

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In a previous paper supporting the theory of intimal herniation as a principal mechanical factor in the production of arteriosclerosis, values for Young's modulus of the ligamentum nuchae were given. These values were compared with values for the human aorta as calculated from the experimental data of Roy (1880) and for those of Mayeda (1934). It was indicated that the aorta is more elastic (in the common usage of the word) than is elastic tissue per se and hence this property is inherent in the architecture of the vessel rather than in the physical character of the individual fibre. (Krafka, 1937.)

It is the purpose of the present paper to analyze further the problem of the histo-physics of the aorta.

In the previous investigation, elongation was measured directly as varying weights were suspended from the piece of tissue. The present series of tests were carried out on a Scott serigraph, an instrument designed to apply increasing force by a shift in the angulation of a track carrying a traveling carriage according to the formula:

$$F = \text{Sin } A \times W$$

where F is the force, A the angle of inclination of the rod on which the carriage rolls, and W the weight of the carriage.

With this instrument, force can be varied from 0 to 250 grams, without changing the direction of pull, in the period of one minute. The elongation is graphically recorded, repetitions of tests are easily made and the constants may be calculated at leisure. The test piece is held firmly at either end by eccentric clamps, and slip, were it to occur, is evident at

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once. This instrument is of the type commonly used in rayon, silk, rubber and cotton industries to test elongation and tensile strength of fibres. Checks on its accuracy are given by Hunter, 1936.

METHODS. For the following series of tests, pieces of tissue were obtained from several sources. Those of the beef came from a local abattoir and were secured shortly after the animals had been killed by the sledgehammer method. All strips were in the machine and elongation records complete within two hours after death. In the initial tests, pieces were bathed in Ringer's solution, but this was found unnecessary when the work was carried out rapidly. Strips of aorta from the dog were secured from the Surgery Department immediately after the dogs had been killed by either air embolism or bleeding. Strips from man were available at autopsies carried out by the Pathology Department twenty-four to forty-eight hours after death.

Standard strips were 10 cm. long and 1 cm. wide. Standard strips were not always available but it will be seen from the formula below that for practical purposes of comparison Young's modulus, M, is corrected for variation in length and cross sectional area. Check tests were made on this point.

Elongation was measured directly from the rectilinear serigraph chart and weighings, required for the estimation of cross sectional area by the pycnometer method, were recorded on a standard work sheet (Krafka, 1937).

Young's modulus, M, was regularly calculated for four weights, 25 grams, 50 grams, 100 grams and 200 grams. Since M is not a constant for all levels of tension for the aorta, the term M^{25} has been adopted to express the value of Young's modulus when a standard piece is stretched longitudinally in the serigraph with a force of 25 grams. M^{50}, M^{100}, etc., represent the moduli when the stretching forces are 50 grams, 100 grams, etc.

The formula for the calculation of M is as follows:

$$M = \frac{W \times L \times 980}{A \times el}$$

where W is the weight in grams, L the length of the piece in centimeters, A the cross sectional area in square centimeters and el the elongation in centimeters; 980 is the factor converting grams into dynes. All values will be expressed in dynes $\times 10^6$ per C^2.

From the standpoint of the physicist, the modulus is considered the principal modulus of the material which is treated as aeolotropic. No attempt was made at the present time to correct for Love's 21 variables nor Poisson's ratio (Love, 1892).

Values for M might have been calculated for any given force level as has been done by other investigators. Such a series would doubtless leave

an impression of extreme accuracy. But at the same time the significant variability would be obscured. The values M^{25}, M^{50}, M^{100} and M^{200} fall well within the significant portions of the curves and hence the above procedure has been generally adopted.

The analysis of the problem arranges itself logically under four separate heads: 1, direct comparison of Young's modulus for the ligamentum nuchae and the aorta of the cow; 2, comparison of values for the aortae of cow, dog and man; 3, analysis of the curved portion of the elongation curve of the aorta; 4, analysis of the straight line portion of the elongation curve of the aorta.

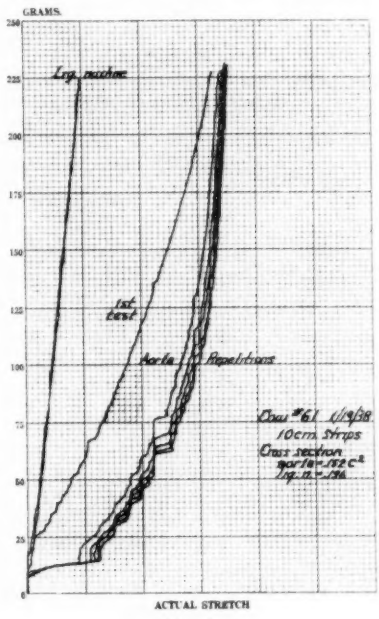

Fig. 1

Direct comparison of Young's modulus for the ligamentum nuchae and the aorta of the cow. Material was obtained from sixteen cows and elongation tests were made for strips of ligamentum nuchae and aorta. A typical curve is presented in figure 1. The curve for the elongation of the ligamentum nuchae is practically a straight line. The calculated values for M are given in table 1 for individual animals and for the averages.

The average value of M shows a general decrease for increasing tension (7.64 to 4.06). The values here established ($2.10–14.39 \times 10^6$) are consistent with those previously published (1.57–5.14). No attempt was made to estimate the age of the individual animals in the present series. Since the techniques employed in the two series, while fundamentally alike, were so different in operation, the results show a very good correlation and speak for a high degree of accuracy in each.

The curve of elongation for the aorta is an exponential curve with the hollow at the 25–100 gram level (fig. 1). Above the hollow, the curve straightens out. The values for M for the aorta are all consistently lower than those for the ligamentum nuchae. If the M^{25} values be momentarily neglected, the general phenomenon appears that M increases for the higher stresses (1.14, 1, 15, 1.46) (table 1). The high modulus for the 25 gram stress is in part due to a small amount of inertia residual in the machine at the beginning of slight angulation. This of course is not appar-

ent in the tests on the less elastic ligamentum nuchae, but the extremely high values at M^{25} may in part be due to this cause.

A significant difference in the elasticity of the aorta and the ligamentum nuchae is brought out by repetition of tests. While repeated tests on the same strip of ligament give superimposed curves, there is a marked difference between the first curve and all subsequent curves for a strip of aorta. The first curve for a strip from a recently killed unanesthetized animal falls about half way between that for the ligament and those run subsequently for the aorta. All tests on the aorta, after the first, show a

TABLE 1

Comparative values of Young's modulus M, for ligamentum nuchae and the aorta as calculated from elongation tests for longitudinal strips as measured by the serigraph. Sixteen cows

ANIMAL NUMBER	M^{25}		M^{50}		M^{100}		M^{200}	
	Lig.	Aorta	Lig.	Aorta	Lig.	Aorta	Lig.	Aorta
1	4.16	3.78	4.16	2.16	4.16	1.51	4.34	1.08
2	4.30	2.05	3.44	1.30	3.20	1.29	3.20	1.90
3	8.50	2.27	3.40	1.82	2.93	1.96	2.72	2.70
4	2.84	3.55	2.10	1.77	2.24	1.42	2.59	1.70
5	3.33	1.83	2.49	1.34	2.28	1.46	2.66	2.09
6	5.41	4.15	4.32	2.07	3.97	1.71	4.12	1.94
7	12.03	2.05	8.02	1.42	4.81	1.48	4.81	1.84
8	14.39	0.79	4.11	0.89	3.00	0.84	2.96	1.00
9	13.96	0.81	13.96	0.24	13.96	0.23	6.20	0.22
10	4.10	1.04	3.28	0.94	3.64	1.15	5.64	1.56
11	8.51	1.22	5.66	0.98	4.25	0.98	3.40	1.08
12	11.46	0.92	7.64	0.81	5.08	0.74	4.16	0.83
13	11.76	0.63	5.88	0.70	5.24	0.74	5.24	0.93
14	3.94	2.65	3.50	1.41	3.15	1.29	3.42	1.24
15			6.82	0.89	6.82	1.02	5.45	1.30
16					4.98	1.30		
Dynes $\times 10^6$ per C^2	7.64	1.75	5.21	1.14	4.60	1.15	4.06	1.46

permanent set and may be repeated as many as thirty times without significant variation. The explanation of this phenomenon will be considered in the section of this paper dealing with an analysis of the lower half of the curve;—namely, with the rôle of smooth muscle.

At this point the conclusion may be drawn that the aorta is more elastic than is elastic tissue per se, and the hypothesis again defended that this quality is a function of fibre arrangement rather than the physical property of the so called "vital rubber" of the aorta.

Comparison of Young's modulus for the aortae of the cow, dog and man.

A considerable number of studies have been made on the elasticity of blood vessels using various animals as the source material, but no investigations are extant that make direct comparisons using identical methods for each species. The following data present measurements on the aortae of sixteen cows, fourteen dogs and fourteen men. Individual curves were all strikingly similar to that shown in figure 1. The basic data for the comparisons are given in table 2.

Two significant factors enter the problem of such a comparison; namely, sclerosis and vessel size. Sclerosis of course is common in other animals than man. In this investigation a beautiful example of calcification was discovered in the aorta of one cow which definitely supports the "branch tug" theory of etiology. The single plaque was crescentic in form and embedded beneath the intima at the point of entrance of the ligamentum arteriosum. In a second specimen, generalized atherosclerosis was observed. Neither of these pathological features seemed to alter the value of M as significantly as sclerosis does in man.

TABLE 2

Average values of Young's modulus for longitudinal strips of the aortae of 16 cows, 14 dogs and 14 men

	M^{50}	M^{100}	M^{200}
Cow	1.14	1.16	1.45
Dog	1.54	1.90	2.67
Man:			
Non-sclerotic	1.88	2.35	4.20
Sclerotic	4.19	5.23	7.47

Among the dogs, sclerosis was met with once. Three small saccular aneurisms were also found but again the elasticity was not specifically affected by either condition.

The data for human aortae are arranged according to age and sclerosis. Of the eight individuals, age 40 years and over, three are nonsclerotic, yet one, age 49, shows a high M, 6.92, as compared to a 6.94 for a sclerotic age 47. One sclerotic specimen from a man 70 years old has a comparatively low M. The general impression obtained from the series is that while a correlation exists between sclerosis and loss of elasticity, the latter is not entirely dependent on the former. A complete analysis of the effect of sclerosis on the three histological elements in the aorta will not be made at this time. The examination of a more extensive series is in progress.

A second factor apparently affecting the value of M for the three species is that of size of the vessel. The thickness of the wall of the cow's aorta may be 8 mm. while that in the dog and man varies from 1 to 2 mm.

Hence while the three are not strictly comparable this factor is partially corrected for in the formula, and except for the sclerotic group in man at the higher tensions, a fair range of similar values is in evidence.

Criticism may be leveled at the strip method of testing based on the fact that some fibres are cut in the removal of the tissue. A test was made on a 10 cm. length of the whole aorta of a macaque monkey to determine the significance of this objection. The values follow: M^{50}, 1.79; M^{100}, 3.18; M^{200}, 5.20. From these values it would appear that the strip method is satisfactory in so far as this factor is concerned.

The average value of M at all levels is consistently higher in the dog than in the cow. A possible explanation may be had in the fact that there is less muscle in the aorta of the dog. The average value for man is higher than for the other two groups, but when the sclerotics are excluded, the value for man is only slightly higher than for the dog (i.e., 1.88–1.54).

From the values of M and the general similarity of the curves for the three species, the general conclusions may be drawn that the problem of elasticity of the aorta is the same for cow, dog and man.

An analysis of the curved portion of the elongation curve of the aorta. Direct attempts to analyze the elongation curve for the aorta are few in number. There is however considerable theoretic speculation as to the rôles played by the separate histological elements, elastic fibres, muscle fibres and collagenous fibres. Reuterwahl (1921), Hwiliwitzkaja (1926), Petersen-Giesen (1924), Ranke (1924) and others have attempted to show the effect of muscle by comparison of volume elasticity under various conditions.

Two inherent difficulties have delayed the analysis, namely: 1, failure to recognize the significance of the time and nature of death, and 2, the assumption that Young's modulus is a constant for all levels of force. MacWilliams (1902) has presented data on the first point and data herein included show the relation of the values of Young's modulus to the elongation curve.

The exponential character of the elongation curve is well established, and explanations have been postulated. It is generally assumed that muscle tonus is involved in the lower half of the curve. On this premise, a test on an aortic strip from an animal immediately after a non-anesthetic death, because of muscle tonus, should give a curve different from that of an animal dead for 24–48 hours. Such a difference has been discovered in the present series of tests. The curve obtained for the initial stretching of a strip from a non-anesthetized animal (beef or dog) consistently showed a higher modulus than did subsequent repeated tests on the same strip (fig. 1). Repeated tests gave practically superimposed curves.

Strips from human aortae secured at autopsies 24 to 48 hours after death, failed to show this significant difference.

The average percentage difference between the value of M^{50} for the first test and subsequent tests is 24.7 per cent for cows, and 31.1 per cent for the dogs. This difference may be due to loss in muscle tonus during the first stretching. The percentage difference for man varies widely from 0 to 32.2 per cent with an average of 11 per cent. For six cases the average is only 4.3 per cent. This difference in the behavior of the human strips may be reasonably explained as due to individual differences in muscular relaxation during the time elapsed between death and the tests.

That the difference between the first and subsequent curves is not due to slip is evidenced by the fact that the upper points on the two curves are nearly identical.

That muscle is the element involved in the initial tonus is shown by a series of tests with ephedrine. A typical test is as follows: a strip of aorta from the cow was stretched on 1/13/38 initially and then for several subsequent repetitions. The piece was then removed from the serigraph, soaked in a dilute solution of ephedrine and the same tests again carried

TABLE 3

Values of Young's modulus for an aortic strip from the cow before and after the use of ephedrine

	BEFORE EPHEDRINE		AFTER EPHEDRINE	
	First test	Subsequent test	First test	Subsequent test
M^{25}	5.37	0.537	1.23	0.537
M^{50}	1.73	0.606	1.17	0.690
M^{75}	1.47	0.876	1.16	0.980
M^{100}	1.64	1.512	1.79	1.680

out on the next day. The results on the two days were almost identical. The values are given in table 3.

A similar series of tests on comparable strips of ligamentum nuchae show no difference between the first and second stretchings, nor any effect of ephedrine. Hence it is highly probable that muscle tonus is the major variable in maintaining elasticity in the aorta at pressure levels represented by the curved portion of the serigram.

Two concepts of the musculo-elastic systems of the aorta have been advanced. According to Petersen-Giesen, these two histological elements are independent of each other. Ranke argues that their actions are correlated. The above data give tentative evidence significant in this problem, since theoretically it is possible to give a quantitative estimate of the force developed as resistive tension by the muscle of the aorta. By subtracting M for subsequent tests from M for the initial test, a value for muscle tension may be considered.

This muscle factor or difference value of M^{100} for first and subsequent

tests on the aorta of the cow has a value of 0.299. If now, the musculature of the aorta is considered to make up approximately ten per cent of the vessel wall, Young's modulus corrected for cross sectional area should be multiplied by ten. This would give a value 2.99, a fair approach to the value 8.06 established for the elongation of smooth muscle taken from the intestinal wall as shown in table 4.

A highly important step in the analysis of this problem is a comparison of M for the aorta and M for strips of smooth muscle. While the testing of the elasticity of smooth muscle has long been considered unsatisfactory, results obtained should be put on record as basic for either confirmation or

TABLE 4

Average values of Young's modulus for strips of smooth muscle from the taenia coli of 9 men, 1 monkey and 1 calf

M^{25}	M^{50}	M^{100}	M^{200}
6.99	6.38	8.06	11.21

TABLE 5

Comparative values of M for the aorta and taenia coli. Human

CASE NUMBER	AGE		M^{25}	M^{50}	M^{100}	M^{200}
79	10 mo.	Aorta	1.58	2.37	3.79	5.80
		Taenia	13.77	13.77	18.37	24.49
81	22 yr.	Aorta	1.68	1.56	2.19	3.72
		Taenia	2.35	3.72	6.42	18.08
77	60 yr.	Aorta	2.32	2.32	2.72	3.58
		Taenia	1.62	2.59	3.96	6.48
78	63 yr.	Aorta	1.82	2.09	2.93	4.19
		Taenia	16.01	10.67	9.15	8.00

rejection. All previous investigators have been hesitant to give a quantitative expression to their observations because of a number of uncontrolled factors. But until some definite data are set down, no advance in the knowledge of muscle elasticity can develop. The following measured values of Young's modulus are offered for nine strips of intestinal musculature taken from man, one from a calf and one from a monkey.

Of more interest to the problem at hand is a direct comparison of M for the aorta and M for the taenia coli taken from the same individual. The data are given in table 5.

It is at once apparent that the aorta is much more elastic (in the com-

mon usage of the term) than is smooth muscle. While the state of tonus, rigor, etc., is not here established, theoretically at least these should be the same for both the aorta and the intestine. Hence a tentative conclusion may be reached from these values, namely, that smooth muscle does have the capacity to impart by contraction a high modulus to the aorta.

The most surprising fact that appears from these comparative data is the similarity between the values of M for smooth muscle and M for elastic tissue per se. Such a comparison may be made by consulting table 1 for the values of M for the ligamentum nuchae, and table 4 for taenia coli. Thus M^{25} for smooth muscle is 6.99 and for elastic tissue 7.64.

The striking agreement between the two sets of values would seem to indicate that the musculo-elastic system of the aorta is very closely integrated and that the low value for the aorta (less than either muscle or elastic tissue) is due, within certain ranges, to the netlike arrangement of the histological elements which compose its wall. This would permit a wide adaptibility to a variety of pressure and volume changes in a flexible hydrodynamic system. The evidence supports the contention of Hwilwitzkaja (1926) that the elastic fibres are brought into play only when the muscle fibres are relaxed.

An analysis of the straight line portion of the elongation curve of the aorta. A consideration of the third histological element in the aortic wall is highly significant in the problem of elasticity since it is generally accepted that white fibres are non-elastic. Yet no direct comparisons of the moduli for the aorta and for tendons have been systematically presented, where a single system of testing was involved.

Preliminary tests on tendons showed straight line curves with high moduli, while the curve for the aorta is a typical geometric curve with the hollow at the 50 to 100 gram tension level. While the lower half of the aortic curve has little in common with that of the tendon, comparisons in the straight line portion establish definite relationships for the elastic moduli, which favor the view long held by physiologists that at the upper pressure stresses, collagenous fibres are brought into play.

In order to make rational comparisons between the upper portions of the aortic curve and the curve for tendons, a new concept of calculation must be introduced, which will negative the effect of the special factors operative in the lower half of the curve. Thus while the term M^{100} is the modulus when a standard piece is subjected to an initial tension of 100 grams, the term $M^{200-100}$ is the modulus calculated for the additional stretch produced by 100 grams on a piece already under a tension of 100 grams. This method will be referred to as the tension increment method. It is justified on the ground that it deals more specifically with the three separate histological elements that make up such an aeolotropic structure

as the aorta. A comparison of the values of M^{100} and $M^{200-100}$ is given in table 6.

From this table it is clear that the modulus as calculated by the incremental method for 100 grams tension when the piece is under an initial tension of 100 grams is from 2 to 9 times that for the 100 grams calculated by the direct method from an initial condition of no stretch.

For the aorta of the cow the difference is not as conspicuous as it is for the other two species. This may be associated with the relative amount of musculature present in the cow or possibly with the size of stroke volume.

TABLE 6

Average values of Young's modulus for the aorta calculated by the direct method for the 'first 100 grams' tension, and by the incremental method ($M^{200-100}$) for the second 100 grams' tension

	M^{100}	$M^{200-100}$
Cow	1.15	2.69
Dog	2.16	18.14
Man	3.43	16.71

TABLE 7

Comparative values of $M^{200-100}$ for the aorta and M^{100} for tendons, etc.

AORTA OF	$M^{200-100}$	M^{100}	
Dog	18.14	28.50	Linea alba of dog
Man	16.71	61.73	Dura
Cow	2.69	55.53	Dura
		43.86	Plantar tendon
		40.50	Tendo Achilles
		85.30	Central tendon diaphragm
		27.36	Central tendon
		18.51	Central tendon calf
		178.68	Capsule of eye beef

The same phenomenon of decrease in elasticity however is found in the cow if a comparison is made between M^{500} and $M^{1000-500}$. The values are M^{500}, 3.26; $M^{1000-500}$, 12.65. The limit of muscle and elastic tissue elasticity has not been reached in the cow at the 100 gram level.

Presumptive evidence that the collagenous fibres are responsible for the change in modulus at the higher tensions is to be had when a comparison is made between the values of $M^{200-100}$ for the aorta and M^{100} for various collagenous tissues such as tendons and ligaments. Table 7 gives the data necessary for such a comparison.

While the values of $M^{200-100}$ for the aorta do not reach those for the

more definitely fibrous tissues, they do suggest the entrance of collagenous fibres as check fibres limiting stretch.

In order to investigate this relationship further, the initial work of Roy was repeated in a series of tests on the elasticity of the aorta intact, the stripped media and the stripped adventitia. It will be seen from the following table 8 that the value for the stripped adventitia is consistent with the values for the intact aorta at the higher stresses.

Another approach to the problem was developed by making tests on the aorta, before and after putrefaction. The immediate effect of putrefaction

TABLE 8

Comparative values of M^{100} for the aorta intact, the stripped media and the stripped adventitia

AORTA INTACT	STRIPPED MEDIA	STRIPPED ADVENTITIA
1.15	1.02	17.57

TABLE 9

Comparative values of M for a single strip of the aorta before and after putrefaction

	BEFORE PUTREFACTION	AFTER SEVEN DAYS OF PUTREFACTION	AFTER TWENTY-FOUR DAYS OF PUTREFACTION
M^{25}	1.57	0.55	0.22
M^{50}	1.37	0.61	0.43
M^{75}	1.36	0.72	0.62
M^{100}	1.37	0.93	0.82
M^{200}	2.04	1.79	1.52
Same data calculated by tension increment method			
M^{50-25}	2.16	0.83	16.53
M^{75-50}	1.53	3.76	16.69
M^{100-75}	1.76	5.15	16.85
$M^{200-100}$	8.92	10.52	19.14

is of course to lower the values of M for all levels, when calculated from a point of no-stretch, i.e., M^{25} before putrefaction is 1.57; after 7 days, 0.55; after 24 days 0.22. If, however, the date is calculated by the tension increment method the values materially increase and reach those for fibrous tissue per se. Values by both methods of calculations are given in table 9.

Since the collagenous fibres are the last to disintegrate by putrefaction, as was established by histological section of the putrefying pieces, the conclusion may again be drawn that these fibres are responsible for the high moduli.

Before the discussion of this topic is terminated, a comparison of the

values of Young's modulus for tendons, etc., as here established and those found by other investigators is in order. Haycraft, 1904, reviewed the subject of elasticity of various animal tissues, but his work is disappointing in that no quantitative values are given. I have calculated the value of M^{100} for the tendon of the musculus extensor digitorum communis from the data of Reuterwahl and get the low value of 2.61×10^6. I have calculated the value of M^{500} from the data of Gratz and Blackberg for the tendo Achilles of the sheep and get 5.47×10^6 when I convert his grams to dynes. But by calculating directly from his experimental data the value is 66818×10^6.

It is evident that all workers in the field of elasticity must agree upon a common method of calculation and on the terms in which Young's modulus is to be expressed. I believe after dealing with a wide range of material, that the adoption of the standard text-book formula is best adapted to bring clarity to the studies in comparative elongation of various tissues.

$$M = \frac{F \times L \times 980}{a \times el}$$

In summary of the four sections of this paper it may be pointed out that the problem of elasticity of the aorta is the same for all species; that the aorta is more elastic than elastic tissue per se; that an exponential curve is established and that the separate portions of this curve must be independently analyzed. The general conclusions may be drawn that the hollow portion of the curve is due to 1, architecture of the aorta; 2, variable elastic moduli dependent upon the muscle tonus. The straight line portion of the curve is dependent upon the high moduli of the white fibres.

The relationship of these various factors to the problem of blood pressure will be discussed in a subsequent paper. The data involve rather complex engineering problems. For the present, it is of interest to state that calculations based upon stress and elongation, vessel size, thickness of the wall, stroke volume, etc. fix the force equivalent of normal systolic pressure at 142 grams and the diastolic at the 74 gram level on the serigram. Hence the foregoing data are in keeping with the accepted idea that muscle operates at the normal blood pressure levels and connective tissue in hypertension.

SUMMARY OF DATA

1. Values of Young's modulus are given for the ligamentum nuchae and the aortae of sixteen cows, as calculated for measurements on a Scott's serigraph.

2. For a single animal the comparative value of M is always significantly lower for the aorta than that for the ligament.

3. The average value for the aorta ($M^{100} = 1.15 \times 10^6$) is less than that for the ligament ($M^{100} = 4.60 \times 10^6$).

4. The moduli for the ligament decrease slightly for increasing stresses; M^{50}, 5.21; M^{100}, 4.60; M^{200}, 4.06.

5. The elongation curve for the ligamentum nuchae is a straight line.

6. The moduli for the aorta increase for increasing stresses; M^{50}, 1.14; M^{100}, 1.15; M^{200}, 1.46.

7. The elongation curve for the aorta is an exponential curve.

8. The values for Young's modulus are given for the aortae of sixteen cows, fourteen dogs and fourteen men. The latter include nine normals and five sclerotics.

9. Comparison is made between the values of M for the initial stretching of a strip and the subsequent stretchings.

10. Significant differences were found which may be correlated with the length of time elapsed between death and test, and with the nature of the lethal agent. Tests before and after ephedrine are presented.

11. Values of M for smooth muscle from the intestine are recorded as a basis for more critical studies on tonus.

12. Direct comparison is made between M for the taenia coli and M for the aorta of the same individual. M for taenia is always the higher.

13. Comparison is made between M for smooth muscle and M for the elastic tissue of the ligamentum nuchae. The values are strikingly alike.

14. The relative rôles of muscle and elastic tissue in maintaining the elasticity of the aorta are discussed.

15. Comparisons are made between M for the lower hollow portion of the elongation curve of the aorta and M for the upper straight line portion of the curve. An incremental method of calculation is introduced and its significance discussed.

16. Comparisons are made between M for the straight line portion of the aortic curve and M for ligaments and tendons. The values of the former approach those of the latter.

17. Comparisons are made between M for the aorta, before and after putrefaction, as indicating the effect of white fibres on high moduli.

18. The data are discussed on the hypothesis that collagenous fibres are responsible for the straight line character of the upper half of the curve.

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EFFECT OF LESIONS IN THE MEDIAL GENICULATE BODIES UPON HEARING IN THE CAT

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It has been reported by Culler (4) and by Stevens, Davis, and Lurie (8), that the several segments of the cochlea respond differentially to acoustic stimuli, the higher frequencies being resonant in the basal coils, and the lower near the apex. Culler, Willmann, and Mettler (5) have made a preliminary report, tentatively confirming these electrical results with hearing-tests, by use of the conditioned-response technique, after localized lesions in the cochlea.

The anatomical studies of Lorente de Nó (6) on the central connections of the cochlear nerve demonstrate that its endings in the cochlear nucleus form a projection of the spiral ganglion of Corti. Walker (9) has shown in the monkey, by the method of retrograde cell-degeneration, that localized areas of the internal geniculate body degenerate following localized lesions of the temporal cortex.

Functional evidence on localization of tone at any level above the cochlea is conspicuously lacking. Meagre clinical records indicate the possibility of partial tonal deafness in consequence of localized injury to the temporal cortex. The medial geniculates being the final way-station from cochlea to cortex, they provide an ideal site for determining by what pathways cochlear impulses are transmitted to the brain.

MATERIALS AND METHODS. Young adult cats have been used throughout this study, being chosen for size and age without regard to sex. In general the procedure has been as follows: the cat is conditioned and thresholds are obtained for several frequencies, adequately covering the audible range; localized electrolytic lesions are effected in the medial geniculate bodies; the cat is retested to determine losses in hearing; and, finally, the cat is killed and the brain prepared for histological study.

The cats were conditioned and tested, in the way described by Brogden and Culler (1), at seven frequencies, representing the octaves from 125 to 8000 c.p.s. The animals were routinely tested each day until five or six

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consistent limens (a series of tests being deemed "consistent" when the standard error of their mean does not exceed 1 decibel) for each frequency had been obtained. They were then operated with aseptic precautions under Nembutal anesthesia. Bilateral (symmetrical) electrolytic lesions were made in the medial geniculate bodies. The electrode used consisted of a sharp tungsten wire, insulated except at the tip with baked enamel; a Horsley-Clarke stereotaxic instrument was used in placing the tip at the desired point within the geniculates. Cautery of a sharply localized area around the electrode-tip was effected with a pentode-circuit which insured a constant current despite any change in resistance such as may result from accumulation of oxidation-products. The inactive electrode consisted merely of a clip-lead fastened to the edge of the scalp incision or the temporal muscle. A current of 3 m. a. passed for 20 to 30 seconds was found to produce a lesion of approximately the desired size (1 mm. diameter).

Certain modifications of the usual Horsley-Clarke technique were introduced to meet the peculiar requirements of these experiments. The usual type of ear-plug (used to fix the instrument at the inter-aural line) penetrates the tympanum and rests against the medial wall of the tympanic bulla; post-operative tests of hearing are thus made impossible. A shorter plug was therefore developed, which, while capable of being securely anchored in the bony meatus, was too short to damage the tympanum.

It was also found desirable to avoid unnecessary damage to the skull in making an opening through which to insert the electrode. Consequently, a special holder for a dental drill was developed, so constructed that it could be substituted for the electrode-carrier on the Horsley-Clarke instrument; the drill, being thus centered in the true position, cut a hole just large enough to admit the electrode.

Most of the brains were prepared for histological examination according to the Marchi-method. Some few were fixed in formalin, and subsequently stained by the Weil technique for myelin sheath, alternate sections being stained with cresyl echt-violett for cell-bodies. All brains were embedded in celloidin and cut at 50 μ. Marchi studies of the brains are being conducted as a separate enterprise.

Results. Pre-operative test-results represent in each case the mean of at least *five* daily limen-determinations on each frequency, no animal being operated until the variance of its mean-threshold had dropped to one decibel or less. The post-operative figures are likewise based upon five measures, save for a few cases when the cat became unduly lethargic after operation or displayed other untoward symptoms. In these circumstances no tests were made until the animal was sufficiently recovered to meet our criterion of consistent performance as stated above.

Table 1 shows mean hearing losses at each test frequency for each of twelve animals. Audiograms for the animals described below are as-

sembled into figures 1, 2 and 3, each being self explanatory. The location of a lesion is referred in each case to three planes which intersect at right angles in the center of the geniculate, dividing it into an anterior and a posterior half, a lateral and a medial half, a dorsal and a ventral half.

Comment on individual animals. 1. Cat 1. Daily limens were taken for eight days before and eight after operation; recovery was rapid and the animal remained in good physical condition throughout the test-period. Histological examination showed that the left medial geniculate was almost completely destroyed while the right remained virtually intact.

2. In cat 2 an effort was made to destroy the left medial geniculate completely by means of several lesions. Tests were obtained eight days before and eight after operation, the animal being in good condition

TABLE 1*

CAT	FREQUENCY							C.P.S.
	125	250	500	1000	2000	4000	8000	
1	−10.55			−9.50			−9.54	Decibels
2	−2.8			−9.00			−3.8	Decibels
3	−1.6	+1.2	−1.2	−1.2	−4.8	−16.0	0.0	Decibels
4	−9.2	−12.2	−26.6	−28.3	−21.0	−10.4	−4.8	Decibels
5	−5.2	−10.8	−19.2	−20.8	−18.0	−11.2	−5.5	Decibels
6	−1.8	−5.5	−6.4	−19.2	−21.4	−4.9	−2.2	Decibels
7	−4.7	−4.2	−5.0	−9.0	−13.5	−19.7	−13.0	Decibels
8	−8.0	−8.0	−14.0	−12.0	−19.0	−24.0	−15.0	Decibels
9	−7.6	−8.0	−20.6	−13.0	−12.5	−7.5		Decibels
10	−5.8	−6.2	−15.8	−7.6	−5.0	−4.4	−1.6	Decibels
11	−3.8	−3.2	−4.5	−8.5	−18.9	−12.8	−8.3	Decibels
12	−4.0	−4.0	−3.3	−6.0	−13.0	−22.0	−44.0	Decibels

* The figures given for each animal represent the difference between means of pre- and post-operative limen determinations for each frequency.

throughout. Histological examination revealed that only the posterior half of the left medial geniculate was affected by the lesions, the remainder being unimpaired.

3. With cat 3, as with all succeeding animals, tests were obtained at the seven octaves from 125 to 8000 cycles. Lesions were made bilaterally according to the method set forth above. The animal remained in excellent condition for 24 days after operation and was then dispatched. The lesions proved to be not quite symmetrical, but overlapped an area lying in the antero-dorso-medial region. On the right side, the lesion slightly invaded the lateral geniculate body on its ventral border.

4. Cat 4. Five tests were obtained before operation but only three thereafter. While the cat lived for 12 days after operation, it was so depressed during the last 6 days that testing became impracticable. Lesions

were almost exactly symmetrical and situated in the postero-lateral region of the geniculates at about the center of the vertical axis.

5. Cat 5. The recovery from operation was unusually rapid and the animal remained in excellent condition for two weeks at the end of which time it was dispatched. A slight middle-ear infection on one side was noted at autopsy. It is highly improbable that the infection could have affected the hearing-tests appreciably since it involved only one ear and was apparently of long standing; hence, it may reasonably be assumed to have affected the pre-operative tests in the same degree as the post-operative, if at all. Lesions were symmetrical, being situate in the extreme posterior region of the geniculates at the center of both dorso-ventral and medio-lateral planes of the nucleus.

6. Cat 6. Complete sets of pre- and post-operative tests were obtained for cat 6. Recovery from operation was exceptionally favorable, and it

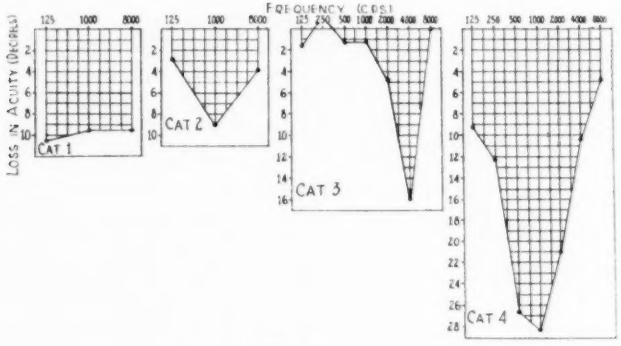

Fig. 1

was still in excellent health when dispatched 14 days after operation. The lesions were not quite symmetrical; but overlapped extensively in the posterior third of the geniculates, slightly dorsad to the center of the dorso-ventral and in the center of the latero-medial plane.

7. Cat 7 made a good recovery from operation, but became quite depressed after 6 days. By this time, 3 sets of consistent tests had been obtained, the animal being alert and responsive. Consequently, on the *6th* day after operation, it seemed advisable to discontinue testing in view both of the concordant limens already obtained and of the cat's increasingly erratic behavior. It was finally dispatched on the *13th* day. Lesions were practically symmetrical, involving nothing but the medial geniculates on either side. They were situate in the extreme anterior end, somewhat dorsad to the center of the dorso-ventral, and in the center of the latero-medial, plane.

8. Cat 8 made a poor recovery and was at no time after operation a satisfactory test-animal; two sets of limens were finally obtained with difficulty. Since the two sets were consistent with each other, they are presented for what they are worth; furthermore, as will appear subsequently, the hearing losses fully accord with those of other animals having similar lesions. The lesions were not precisely symmetrical, overlapping corresponding areas for about half the extent of each. On the right the lesion was situated 2 mm. anterior to center in the antero-posterior, 1 mm. dorsad to center in the dorso-ventral, and on center in the latero-medial plane; that on the left lay approximately 0.5 mm. farther posterior, but in the same relation to the other planes.

9. Cat 9. Although recovery from operation was rapid and the animal was in excellent condition for several days, responding well at all other frequencies, no consistent response could be elicited to the 8000-cycle tone.[2]

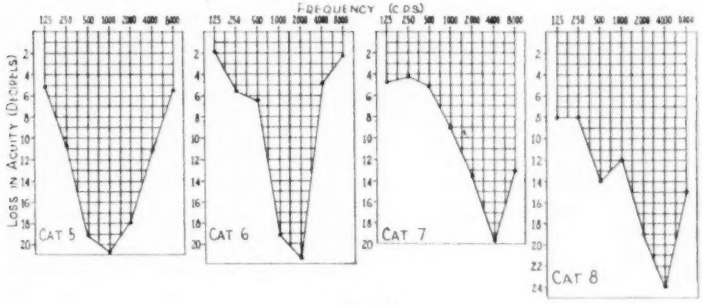

Fig. 2

However, in view of the results on other cats, the loss in acuity at 8000 should have been somewhat less than that at 4000 since the peak loss occurs still farther down the scale at 500. Lesions were symmetrical, being situate at the medial border of each nucleus, 1 mm. dorsad to center of the dorso-ventral, and in the center of the antero-posterior, plane.

10. Cat 10. Lesions were symmetrically placed at the extreme medial border of the geniculates and on the center of the dorso-ventral and antero-posterior planes.

11. Cat 11 recovered promptly from operation and was in unusually good condition for testing throughout the postoperative period of 7 days, at the end of which time it was dispatched. So far as its behavior in the test-equipment is concerned, this animal performed quite as well after

[2] It should be noted that normal cats will ofttimes, for obscure reasons, become erratic at 8000 cycles. Difficulty in obtaining a limen is, therefore, not unique with this animal, nor can it be ascribed to the operative lesion.

operation as before. The lesions were almost exactly symmetrical, situate at the extreme lateral margins of the geniculates, slightly ventrad to center on the dorso-ventral, and about 0.5 to 1.00 mm. anterior to center on the antero-posterior, plane. The optic tract was involved to some extent on both sides where it passes along the lateral border of the medial geniculate.

12. Cat 12. It was at first almost impossible to get a response at 4000 and 8000 cycles, but on the second day after operation, tests were obtained on these two frequencies, the limens remaining fairly consistent in spite of the subject's erratic behavior. It seemed to be very uneasy during tests at those two frequencies, especially 8000, the performance lacking the assurance displayed both before operation, and also after operation at the lower frequencies. The lesions were symmetrical and confined to a small area at the extreme dorsal boundary of the medial geniculates, encroaching to some extent on the lateral geniculates.

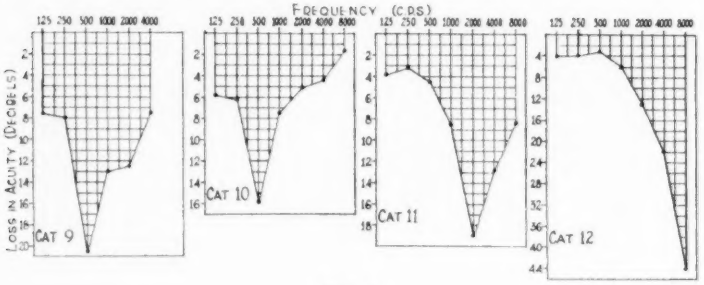

Fig. 3

It should be noted here that one of the cats used in preliminary experiments (not included here), showed almost complete scotoma for 8000 cycles following bilateral lesion of approximately the same area as in cat 12. Unfortunately, the animal had not been tested prior to operation and so the hearing-deficiency cannot with certainty be ascribed to the lesions. However, a strong presumption remains that such was the case, since the audiogram was thus markedly different from the normal.

Discussion. Inspection of the audiograms shows at once that localized lesions in symmetrical areas of the two medial geniculate bodies result in some attenuation of auditory acuity for all frequencies tested, but that the degree of impairment differs widely. The variation is not haphazard, but in all cases conforms to a definite pattern, the loss being greatest at one or two frequencies, graduating down to a minimum at those most distant on the scale from the focal frequency.

The animals described in the preceding section can be divided into sev-

eral groups according to the frequency at which greatest loss in hearing occurs: nos. 3, 7 and 8 at 4000 c.p.s., with lesions in dorso-anterior quadrant; nos. 6 and 11 at 2000 c.p.s., with lesions near the mid-lateral surface, no. 6 being somewhat more postero-medial than no. 11; nos. 4 and 5 at 1000 c.p.s., with lesions just inside the posterior boundary of the nucleus; nos. 9 and 10 at 500 c.p.s., with lesions on the medial surface; no. 12 at 8000 c.p.s., with lesion well up within the dorsal segment.

These facts clearly indicate that impulses concerned with each frequency of the audible range enter and traverse the medial geniculate bodies by separate pathways. In no other way can we account for the differential loss in hearing that results after interruption of limited fractions of the acoustic pathway. This being true, some form of "place" or "resonance" theory is indicated, since it is apparent that each frequency has already, upon reaching the medial geniculate, been routed through its own group of fibers. It is to be presumed that this localization continues to the primary acoustic cortex, in view of the intimate connection between specific areas in the medial geniculate and specific areas in the temporal cortex, as demonstrated by Walker.

The results of the present experiments thus tend to vindicate the principle of localized response of the organ of Corti to tones of given frequency.

In other words, the central acoustic pathway, like the visual and somatic sensory pathways, must be regarded as a projection system. Each localized area of the organ of Corti is projected upon a corresponding area of the primary acoustic nucleus, and, in turn, upon the medial geniculate body, and, probably, upon the primary receptive area of the cortex. Thus, it may be contended that audition is, again like vision and somesthesia, a spatial sense.

It will be noted in nearly all cases that the loss in hearing, though markedly unequal at various frequencies, represents a relatively small percent of the total range of sound-intensity from threshold to limit of endurance, even at the focal frequency. In this connection it should be understood that, while the pre-operative limen for 1000-cycle tone may be given as 90 decibels, that figure does not represent the complete range from weakest audible sound to limit of endurance; it means that the threshold was 90 decibels below the arbitrary zero-level intensity from which our limens are measured, and which is itself many decibels below the limit of endurance. If, for example, as in the case of cat 4, the pre-operative limen at 1000 cycles is 90 (actually 89.6) and the focal loss 28 decibels (actually 28.3), the loss in terms of the original limen is only 31.1 per cent and, in terms of the total intensity-range, still less. The greatest focal loss sustained by any of the animals is in the neighborhood of 50 per cent (cat 12), nearly twice as great as the maximum loss in any of the others.

At first glance, it would seem that if each frequency passes through the geniculate by a separate pathway, severance of that pathway should result in complete deafness or scotoma for the frequency concerned.

If we assume a resonant principle to be operative in the organ of Corti, it must be granted that, while the point of maximum resonance of the basilar membrane would be confined to a small area, it would not be sharply delimited but would diminish gradually in either direction. Consequently, while maximal stimulation would occur at the point of maximal resonance, a certain number of hair cells of adjacent areas must also receive some degree of stimulation, the amount becoming progressively less as the distance from the focal point of resonance increases. Hence, the fibers leading from that point would carry the burden of response to the appropriate frequency, but other fibers from adjacent areas of the organ of Corti would also be excited to some extent. If the pathway be then interrupted at the geniculate body, complete scotoma would occur only if *all* of these fibers were included in the interruption. If any remained intact, some residual hearing for that frequency would remain.

Superficially it might seem that, since the loss in acuity at focal frequency is no greater than it is, the acoustic system must differ from the visual in degree of spatial localization. It is said to be possible to produce sharply delimited visual scotomata by destruction of a small area of one of the higher visual centers. The determination of the extent of scotoma is however, as all clinicians know, a difficult procedure since boundary areas of impaired but not absent vision are usually present. It seems likely that even though a certain small portion of the retina is rendered non-functional through interruption of its central connections, adjacent retinal areas will be stimulated, though in lesser degree than the focus of stimulation. In other words, it is as difficult to imagine a visual stimulus affecting a sharply delimited area of retinal cells as it is to imagine an auditory stimulus impinging on a sharply delimited series of hair cells. Hence, the difference in degree of specificity of localization between vision and audition seems more apparent than real.

Without explaining the more complex auditory phenomena, the present study seeks to determine the manner in which tones are transmitted up the central acoustic pathway, knowledge of which is prerequisite to understanding those phenomena. We can say with assurance that whatever obscure and circuitous paths are followed by impulses from the cochlea after reaching the cortex, they reach that level by way of an orderly spatial projection of the organ of Corti on successively higher centers. In this respect, the plan of the acoustic system is fundamentally similar to that of the visual and somatic sensory systems.

SUMMARY AND CONCLUSIONS

1. Small localized lesions in the medial geniculate bodies produce markedly unequal losses in auditory acuity at the several test-frequencies.

2. Cats with lesions in corresponding areas display the same distribution of hearing-losses. In this way loci for the several frequencies have been established as follows: 8000 cycles, dorsal region; 4000, anterior; 2000, lateral; 1000, posterior; 500, medial. The lower frequencies (250 and 125 cycles) apparently traverse the ventral side. It thus appears that the several loci follow a linear course, beginning with high frequencies in the dorsal section and circling downward to the ventral region where the lower frequencies appear.

3. The functional evidence thus indicates that the medial geniculate body contains a projection of the organ of Corti, each part of the latter being connected by more-or-less discrete bundles of fibers with corresponding areas of the former.

4. Since different frequencies traverse the geniculates by separate pathways, it follows that pitch-discrimination occurs at lower levels (cochlea).

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EFFECT OF ZINC ON INSULIN AND ITS MECHANISM

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Maxwell and Bischoff (1) were first to show that the addition of ferric ions to insulin causes an augmentation of its physiologic effect as measured by blood sugar changes in animals. Since then several investigators (2, 3, 4, 5) have demonstrated that the addition of zinc to insulin causes a retardation and a prolongation of its hypoglycemic effect on blood sugar. No attempt has been made to explain the mechanism of this prolongation.

Clear solutions of insulin for clinical or experimental purposes are usually prepared in a slightly acidified medium. When such a solution is injected subcutaneously the buffer action of the tissues adjusts the acidity of the material injected to about neutrality. Thus the insulin must pass through various stages of hydrogen ion concentration until the reaction at the site of injection is in equilibrium with that of the tissue fluid, whereupon absorption takes place. In order to demonstrate this phenomenon a simple experiment may be performed. One adds a few drops of brom-thymol blue to an acidified solution of insulin and injects a small amount into a clean-shaven area of a rabbit's ear. In about 10 minutes the color of the injected area changes from orange to green and finally to blue. Once the color has become permanently blue, diffusion becomes noticeable which probably corresponds to the initial action of insulin on blood sugar.

In this investigation we demonstrate *A*, the effect of crystalline and amorphous insulin on the blood sugar of rabbits; *B*, the effect of small amounts of zinc added to zinc-free insulin on the blood sugar of rabbits, and *C*, the precipitation of insulin protein by the addition of zinc.

EXPERIMENTAL. *A*. Suitable dilutions of crystalline and unmodified insulin were made so as to contain 2.5 units per 1 cc. In order to present a true picture of the effect of each preparation the "cross-over" method was used; half of the animals received a dose of crystalline insulin and the other half received a dose of the unmodified insulin. One week later, those animals which had received unmodified received crystalline insulin and vice versa. In each instance 1 unit of insulin per kgm. was given subcutaneously. Thirty-three rabbits were used for the test. Blood was withdrawn at

0, 1.5, 3 and 5 hours. The averages of the data obtained are found in figure 1.

B. The preparation of a zinc-free insulin has been previously described (6). From a stock solution of this preparation containing 100 units per 1 cc. a 2 units per 1 cc. dilution was made. This was used as a control. Similar dilutions were then made containing 1, 2 and 4 mgm. of zinc per 1000 units. They were kept in a refrigerator when not in use.

Rabbits that had been starved for 24 hours were used for the test. Five-tenths unit per kilogram of each of the above samples was given subcutaneously to each of 24 rabbits. Blood was withdrawn before and 1.5, 3, 4 and 5 hours after the injection. Averages of blood sugars were then made. The results are shown in figure 2.

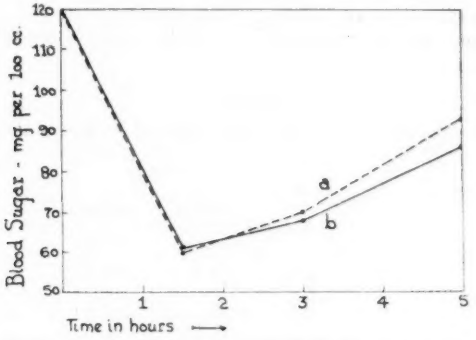

Fig. 1. The effect of crystalline and unmodified insulin on the blood sugar of fasting rabbits. Thirty-three animals were used for each material. One unit per kilogram was given subcutaneously. *a:* amorphous (unmodified) insulin; *b:* crystalline insulin.

C. The use of zinc as zinc hydroxide for the precipitation of proteins has been widely used (7). In this experiment we studied the precipitation of insulin protein with zinc at different acidities. One thousand units of zinc-free insulin were introduced into each of a series of 50 cc. cone-shaped centrifuge tubes. One cubic centimeter of a zinc chloride solution containing 9.5 mgm. zinc was added to each, followed by 2 cc. of sodium acetate buffer. The volume of each sample was brought to exactly 25 cc. The samples were then thoroughly mixed, allowed to stand at room temperature for 2 hours and centrifuged. Nitrogen and zinc determinations were then made on both the supernatant liquids and the precipitates. The nitrogen and zinc in the supernatant liquid were considered to represent the soluble fraction and the amounts of nitrogen and zinc in the precipitate to represent the insoluble fraction of insulin and zinc respectively. The results are recorded in table 1.

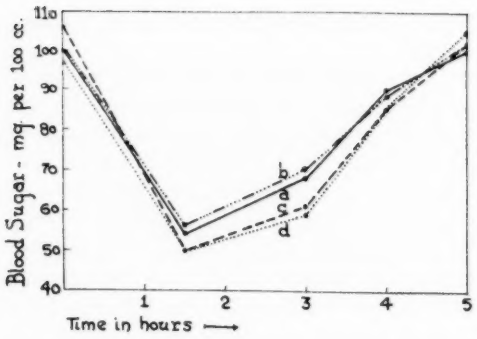

Fig. 2. The effect of added zinc in varying amounts on the blood sugar of 24 fasting rabbits. Five-tenths unit per kilogram was given subcutaneously. *a:* Control, no zinc; *b:* 1.0 mgm. zinc per 1000 units; *c:* 2.0 mgm. zinc per 1000 units; *d:* 4.0 mgm. zinc per 1000 units.

TABLE 1

Precipitation of insulin protein in the presence of zinc, in acetate buffer

MATERIAL	pH	INSULIN		ZINC	
		Soluble	Insoluble	Soluble	Insoluble
		per cent	*per cent*	*per cent*	*per cent*
Insulin + Zinc	4.5	14.5	85.5	92.6	7.37
Zinc	4.5			98.5	1.58
Insulin + Zinc	5.2	9.68	90.4	94.8	5.26
Zinc	5.2			98.0	2.10
Insulin + Zinc	5.8	4.84	95.02	73.8	26.38
Zinc	5.8			99.0	1.05
Insulin + Zinc	6.5	6.45	93.5	73.8	26.38
Zinc	6.5			72.1	27.85
Insulin + Zinc	7.0		100.0	42.1	57.99
Zinc	7.0			44.2	55.8
Insulin + Zinc	7.6	3.22	96.9	36.8	63.01
Zinc	7.6			39.0	61.0

TABLE 2

Adsorption of insulin protein on zinc hydroxide at pH 7.0

Amount of added zinc per 1000 units insulin	Insulin adsorbed
mgm.	*per cent*
1.0	2.0
1.5	12.0
2.0	75.0
2.5	85.0
3.0	90.0
5.0	95.0
10.0	99.0

In the second experiment of this series we studied the minimum amount of zinc required to cause a complete precipitation of 1000 units of insulin protein at pH 7.0. The details are as in the foregoing experiment. The amount of zinc added in each instance and the relative amount of insulin adsorbed at about pH 7.0 are shown in table 2.

Discussion. Since it has been shown that insulin with added zinc has a more prolonged hypoglycemic effect on the blood sugar of animals than insulin without zinc, an attempt was made to determine the minimum amount of zinc required to produce a definite prolongation, and to offer an explanation of the mechanism involved. The data presented in figure 2 show that the insulin containing 1 mgm. of zinc per 1000 units does not exert any greater effect on the blood sugar of rabbits than the same insulin without added zinc. However, when 2 or 4 mgm. of zinc per 1000 units are added to insulin there is a noticeable change in the blood sugar curve (fig. 2). It is to be remembered that the amount of insulin injected was only 0.5 unit per kgm. Had a larger dose been administered the prolonged effect of the added zinc might have been more noticeable but it was our aim to avoid convulsions and permit the animals to return to normal within the 5 hour period.

The prolongation of insulin action by zinc is probably due to the adsorption of insulin protein on insoluble basic zinc salts. In the introductory paragraph we presented a simple experiment in which it was shown that the buffering action of the tissue fluid effects a neutralization of the insulin injected. This is in accord with Donnan's theory of equilibrium and diffusion of ions. Thus when equilibrium is reached at the site of injection the soluble zinc present is transformed to its insoluble form of basic zinc salts. The data presented in tables 1 and 2 show that if zinc is present in sufficient amounts the insulin protein is completely adsorbed at pH 7.0 or thereabout. Thus from a chemical point of view, in order to produce the most efficient and prolonged hypoglycemic curve about 10 mgm. of zinc are required for every 1000 units and any extra zinc need not be added. However, from a physiological point of view, an exchange of ions at the site of injection must be taken into consideration. Since the addition of 1 mgm. zinc per 1000 units does not apparently effect any appreciable precipitation of insulin protein at about neutrality one therefore cannot expect any noticeable change in the blood sugar curve. However, when 2 mgm. of zinc are added to 1000 units of insulin, as the data presented in table 2 show, about 75 per cent of the insulin protein precipitates at pH 7.0. The blood sugar curves of rabbits injected with insulin containing these amounts of added zinc agree with these chemical data.

Kirsner and Bernstein (8), basing their opinion on results obtained with a few animals, have recently reported that in rabbits the effect of crystalline insulin is not only in general more prompt but of less duration than the commercial preparation of insulin. In figure 2 we present the effect of

crystalline and unmodified insulin on the blood sugar curves of 33 rabbits using the "cross-over" method. The crystalline insulin used is identical with the material furnished by this laboratory to Kirsner and Bernstein. Nevertheless we do not propose to draw any conclusion other than that the duration of the blood sugar curve of rabbits injected with crystalline insulin is at least as prolonged as that of rabbits injected with an equal amount of unmodified insulin. Kirsner and Bernstein used their few animals at intervals of two days, switching from one kind of insulin to another. The customary technique is to use rabbits that have been starved for 24 hours, at intervals of one week, and to administer doses of insulin proportional to the body weight. Blatherwick and co-workers (5) using the same crystalline insulin in rats noted a significant difference in the duration of hypoglycemic effect of crystalline and unmodified insulin, with a slight prolongation of the hypoglycemia following crystalline insulin.

Significant are the results obtained by the various laboratories (9) who coöperated on the standardization of the Crystalline Insulin Standard. Although the averages of their results have no bearing whatever on the question of whether or not crystalline insulin has a more prolonged effect on the blood sugar of animals than unmodified insulin, nevertheless one notes a considerable difference between the value of the Crystalline Insulin Standard obtained by the rabbit method and the mouse method of assay. By the rabbit method, 1 mgm. of the Crystalline Insulin Standard assayed at 21.7 International Units and by the mouse method, 25.2. The 14 per cent difference between the two methods of assay is beyond any experimental error. In the mouse method of assay one relies on the incidence of convulsions while in the rabbit method the criterion is the fall in blood sugar. In evaluating the Crystalline Insulin Standard an amorphous insulin standard of 8 units per 1 mgm. was used. Since the comparison was made between a crystalline and an amorphous preparation of insulin, the difference in results may be attributed to a difference in chemical properties, or to the presence of unknown substances in the amorphous insulin. Anyone familiar with the properties of amino acids knows that the solubility of certain amino acids in water is quite different when dissolved singly than when dissolved in the presence of other amino acids. Proteins behave in a similar manner. The solubility of crystalline insulin is not identical with that of unmodified insulin as can be seen from the curve in figure 3. Owing to its solubility and to its freedom from associated proteins crystalline insulin possibly exerts a different effect on blood sugar of animals and of humans.

In their clinical investigations, Rabinowitch and co-workers (10) observed that crystalline insulin has a more prolonged hypoglycemic action than unmodified insulin. This prolonged action was explained on the basis of

the zinc associated with crystalline insulin. The amount of zinc in the crystalline material they used in their investigation was at no time in excess of 0.9 mgm. zinc per 1000 units. Altshuler and Leiser (11) who had previously noted a prolonged action of crystalline insulin investigated clinically the effect of the addition of 1 mgm. of zinc to insulin and were unable to confirm Rabinowitch and co-workers' theory. The chemical data and our results on rabbits, presented in this paper, clearly demonstrate that the addition of 1 mgm. of zinc per 1000 units does not augment the physiological response of animals to insulin. Since crystalline insulin as it is actually prepared does not contain more than 0.04 mgm. zinc per 100 units, it is highly improbable that its hypoglycemic effect on blood sugar is influenced by this small amount of zinc. On the other hand any difference in physiologic response that may be observed between crystalline and

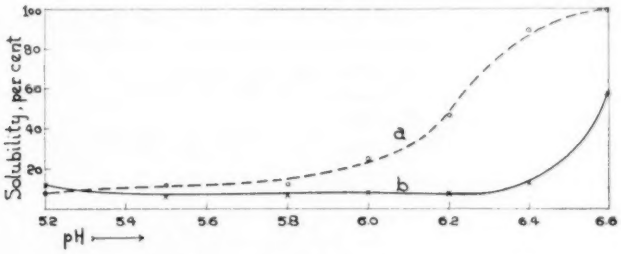

Fig. 3. The solubility of crystalline and amorphous (unmodified) insulin in the pH range 5.2–6.6 *a:* amorphous (unmodified) insulin; *b:* crystalline insulin.

amorphous insulin could be logically attributed to a difference in solubility, and to the purity of crystalline insulin.

SUMMARY

Studies were made of the effect on the blood sugar of fasting rabbits of crystalline insulin, unmodified insulin and insulin to which known amounts of zinc had been added. The data presented show that 1, the hypoglycemic effect of crystalline insulin is at least as prolonged as that of the unmodified; 2, the addition of 1 mgm. zinc per 1000 units of insulin does not augment the physiological response, while 3, the addition of 2 mgm. zinc or more produces a pronounced augmentative effect.

The experimental evidence presented shows that insulin protein is adsorbed on basic zinc salts at about neutrality. This probably explains the prolongation of hypoglycemia produced by insulin with added zinc.

Crystalline insulin is shown to produce a different solubility curve from that of amorphous insulin.

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MAINTENANCE OF PREGNANCY BY PROGESTERONE IN RABBITS CASTRATED ON THE 11TH DAY

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In 1929 Corner and Allen described a method for the preparation of corpus luteum extracts which substituted for the corpus luteum insofar as its functions were known. These extracts produced progestational changes in the endometrium of recently castrated, sexually mature rabbits and, when the castration was done 18 hours after mating at a time when the embryos were already in the tubes, the pregnancy was maintained to term in an essentially normal manner, provided adequate amounts of the extract were given (Allen and Corner, 1930). Since that time one of the active principles in the extracts, progesterone, has been isolated in pure form and it has been shown to possess at least four of the properties known to be inherent in the original extracts, i.e., it produces progestational changes in the endometrium (Wintersteiner and Allen, 1934), inhibition of the action of pituitrin on the myometrium in vitro (Makepeace, Corner, and Allen, 1936), inhibition of uterine motility in vivo (Allen and Reynolds, 1935), and suppression of menstruation (Corner and Allen, 1936).

The maintenance of pregnancy in castrated animals has not, however, been successfully accomplished with progesterone (Allen and Heckel, 1937). We have shown (Allen, 1937) that estrogenic hormone as well as progesterone is necessary if normal progestational changes are to be obtained in rabbits castrated 18 hours after mating. It was decided, therefore, to attempt to maintain pregnancy with progesterone in animals castrated on the 11th day following mating, i.e., after implantation of the embryos had occurred.

The experiments reported in this paper include only the attempts made to maintain pregnancy in rabbits castrated on the 11th day. The results obtained indicate that progesterone alone in adequate dosage will maintain pregnancy in a normal manner after the 11th day. The factors involved in securing successful implantation in animals castrated 18 hours after mating are not yet fully understood.

Pregnant rabbits were castrated on the 11th day and then given daily injections of estrin-free corpus luteum extracts or progesterone, and the

progress of the pregnancy determined by exploratory laparotomy, palpation, or necropsy. Since the amount of hormone necessary was unknown, 1.0 rb.u. daily was given to animals 2 and 3. One developed a hernia and died on the 13th day, and the other was subjected to laparotomy on the 18th day. In both, the implantations were resorbing. In the one explored on the 18th day, the implantation was abnormally small but the pregnancy had obviously been sustained for a short time, since in another

TABLE 1

Effect of estrin-free progestin and progesterone on the maintenance of pregnancy in rabbits castrated on the 11th day

RABBIT NUMBER	PROGESTERONE GIVEN DAILY*	PERIOD OF INJECTIONS	RESULTS
	rb.u.		
1	0		Resorbing on 16th day
2	1.0	11th–17th days inc.	Resorbing on 18th day
3	1.0	11th–12th days inc.	Resorbing on 13th day
4	2.0	11th–26th days inc.	Normal on 16th day (palpation) ? resorbing on 21st day (palpation) ? delivery between 21st and 28th days
5	2.0	11th–20th days inc.	Normal on 16th day (laparotomy). Died 21st day, peritonitis
6	2.0	11th–24th days inc.	Normal on 16th day (laparotomy) Normal on 21st day (laparotomy) Died in labor on 25th day, peritonitis. Fetuses not macerated
7	2.0	11th–19th days inc.	? Normal on 16th day (palpation). All resorbing on 20th day
8	2.0	11th–15th days inc.	Delivered 6 normal living fetuses on 31st day; 4 fetuses successfully nursed
	4.0	16th–28th days inc.	
9	2.0	11th–15th days inc.	Delivered 2 living and 4 dead but otherwise normal fetuses on 32nd day. Living fetuses not nursed
	4.0	16th–28th days inc.	
10	2.0	11th–15th days inc.	Died suddenly on 25th day. Fetuses all normal. Cause of death undetermined
	4.0	16th–25th days inc.	
11	2.0	11th–15th days inc.	Delivery of 6 dead postmature fetuses on the 34th and 35th days
	4.0	16th–31st days inc.	

* Rabbits 7, 8, 9, 10 and 11 received synthetic progesterone kindly supplied by the Schering Corp.; 1 rb. u. is equal to 1.0 mgm. of progesterone.

animal castrated on the 11th day but receiving no injection, the implantations were barely visible on the 16th day. A large series of controls receiving no hormone was not made, because it is well established that castration in the rabbit at this time results in resorption. From these few observations, it is evident that 1.0 rb.u. daily is not sufficient to maintain pregnancy.

The next group consists of eight animals receiving 2.0 rb.u. daily. Two

of these were subjected to laparotomy on the 16th day (nos. 5 and 6), and in both the embryos were normally implanted. In so far as could be determined by abdominal palpation of the other six animals, the implantations were thought to be normal. In four (nos. 8, 9, 10, 11) the subsequent course of the pregnancy proved that the gestation sacs were normal on the 16th day. In the other two (nos. 4 and 7) it cannot be stated with certainty that the pregnancies were normal, since in one premature delivery may have occurred, and in the other all embryos were being resorbed on the 20th day. The results do show that 2.0 rb.u. daily are adequate, in most if not all cases, to maintain pregnancy between the 11th and 16th days.

The next question to be answered was whether 2.0 rb.u. of progesterone daily were adequate after the 16th day. Four animals were given this dose (nos. 4, 5, 6, 7). In only one, no. 6, was the pregnancy normal on the 21st day, and in this one pregnancy did not continue in a normal manner, since she died in labor on the 25th day. Another one, no. 5, died on the 21st day with evidence of peritonitis. Some of the fetuses had evidently lived to about the 20th day. The other two were the ones mentioned above (nos. 4 and 7), one having resorbing embryos on the 20th day, and the other possibly delivering prematurely. These results show that, while 2 rb.u. daily are adequate to maintain pregnancy from the 11th to the 16th days, they are not adequate from the 16th to 20th days.

The next group of four animals received 2.0 mgm. of progesterone daily from the 11th to 15th days. On the 16th day the gestation sacs were of normal size as determined by palpation. The amount of hormone was increased to 4.0 mgm. daily and given at that level for the remainder of the period of injections. One animal died suddenly on the 25th day despite continued injections. No cause for death was found at autopsy. The fetuses were well developed and showed no signs of maceration, so that it seems probable that their death occurred little if any before the mother's. Two animals were injected until the 28th day. One delivered six living, fully mature fetuses on the 31st day. The other delivered two living and four dead but normally developed fetuses on the 32nd day. The fourth one of the group was injected for the last time on the 31st day. Inspection of the abdomen showed the fetuses to be active on the 32nd and 33rd days. On the 34th day, five dead fetuses were delivered and early on the 35th day one more was delivered. All fetuses were post-mature, the crown rump length being from 120 to 140 mm. None showed any signs of maceration. In this group, three of four animals carried the fetuses in a normal manner and in one the fetuses became post-mature as a result of continued injections.

Discussion. The experiments reported here have considerable significance regarding the function of the ovary during pregnancy in the rabbit. Since the ovaries were removed on the 11th day, and the preg-

nancies were maintained in a normal manner when only crystalline, synthetic progesterone was given, it is manifest that the ovaries need produce nothing other than progesterone after the 11th day to maintain pregnancy under normal conditions.

The part which the placenta plays during pregnancy is partially clarified by these experiments. During the latter third of pregnancy it is known that several changes occur which can not be produced in the cas-

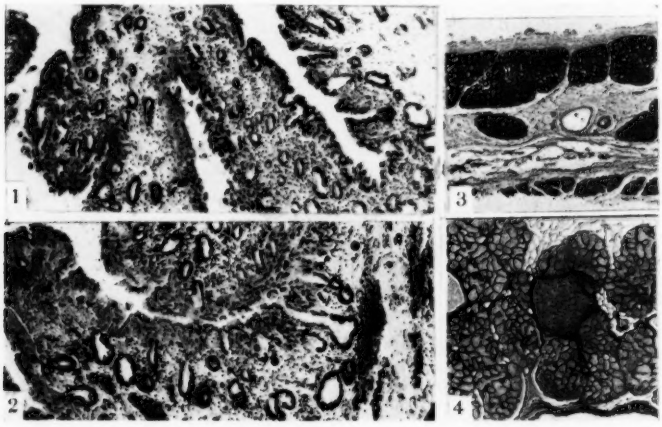

Fig. 1. Endometrium from the sterile uterine horn of a unilaterally pregnant rabbit obtained on the 25th day of pregnancy. × 50.

Fig. 2. Endometrium from the sterile horn of a unilaterally pregnant rabbit (No. 8) castrated on the 11th day of pregnancy and given progesterone daily thereafter. Fetuses were normal on the 25th day when the section was obtained. The late pregnancy alterations are indistinguishable from those of normal pregnancy (fig. 1). × 50.

Fig. 3. Mammary gland from normally pregnant rabbit obtained on the 25th day. × 15.

Fig. 4. Mammary gland from a rabbit in which pregnancy was maintained with progesterone (No. 8). The alveoli are more distended with milk than those of the gland from the normal pregnancy (fig. 3) but this is of little significance since some normal animals show distention of the alveoli on the 25th day of pregnancy. × 15.

trated animal by the injection of progesterone alone (Allen, 1937). The endometrium becomes more edematous and the surface epithelium becomes stratified, giving a picture which is just as characteristic of late pregnancy as the progestational changes are of early pregnancy (fig. 1); the sterile horn in unilaterally pregnant animals grows considerably, becomes much shorter and thicker, and usually appears more cyanotic than during early pregnancy or pseudopregnancy; the mammary glands thicken and between the 25th and 27th days the alveoli become rapidly distended with

milk (fig. 3). None of these late pregnancy changes can be produced in the castrated rabbit by progesterone. Consequently, when the pregnancy is maintained by progesterone, it is of interest to know whether these late pregnancy changes are produced. To ascertain the condition of the endometrium, one animal, in which the pregnancy was confined to one horn of the uterus by resection of one tube, was subjected to operation on the 25th day and a segment of the sterile horn and a small piece of mammary gland removed. The muscle failed to respond to pituitrin *in vitro*, the endometrium showed typical late pregnancy changes (fig. 2), and the mammary gland was thick and the alveoli were filled with milk (fig. 4). In this animal, therefore, the uterus and mammary glands were indistinguishable from those of normal pregnancy. In the other two animals the uteri were not biopsied but the mammary glands became very thick and produced copious milk. It is apparent, therefore, that the products of conception are essential for the production of these changes characteristic of late pregnancy, and that the ovary need contribute nothing other than progesterone. The experiments reported here, of course, do not show whether the placenta alone is responsible, but Klein (1933) has shown that the endometrial changes occur when the fetuses are removed but the placentas retained, provided, of course, that the ovaries are present. It seems logical to conclude, therefore, that the late pregnancy changes are brought about by the combined action of progesterone (produced either in part or entirely by the ovaries) and some other hormone, probably an estrogen (Allen, 1937), elaborated by the placenta.

CONCLUSIONS

Pregnancy can be maintained to term in rabbits castrated on the 11th day after mating providing 2.0 mgm. of progesterone are given daily from the 11th to 15th days inclusive and 4.0 mgm. daily from the 16th to 28th days inclusive. Under these circumstances the same changes occur in the uterus and mammary glands as in normal pregnancy.

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EASE OF BODY HEAT LOSS AS A BASIC DEVELOPMENTAL AND FUNCTIONAL FACTOR IN WARM-BLOODED ANIMALS

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Ease of body heat loss seems to be a basically important factor in determining general metabolic level of warm-blooded species and rate of development in the young. In several previous papers (1) we have shown this to be true for white mice kept under artificially controlled conditions, as well as for man in different natural habitats. Such findings are not at all surprising, since the energy for all body functions is derived from combustion. As an energy-transforming machine the body may be more efficient than most inanimate mechanisms, but even so it is only about 20 per cent efficient in the performance of muscular work (2). The remaining 80 per cent must be dissipated, since the body is very sensitive to the accumulation of this waste heat. The ease with which it can be dissipated becomes an important factor in determining the metabolic rate allowed the individual.

We have shown that this principle applies to such basic functions as rate of growth and development, onset of sexual activity and fertility, and ability to utilize liver glycogen in response to chilling. Moist heat brings about a general metabolic depression, while a cool environment stimulates. Control of body heat loss in past studies was carried out through air-conditioning, which left the possibility that depression might have resulted from breathing the warm moist air, or stimulation might have come from breathing the cooler air. Now, however, we have repeated the studies with heat loss controlled through radiant channels, and have similar findings to report. Radiant heat depresses just as does a warm moist atmosphere, even though the animals receiving the radiant heat live in an atmosphere of cool air. And the depressing effects of a warm moist atmosphere can be overcome completely by facilitating body heat loss through radiant channels alone. The biologic and practical significance of these findings will be discussed in the closing paragraphs.

EXPERIMENTAL CONDITIONS. Experimental rooms were set up within larger laboratory rooms and equipped to cover the desired conditions. In *room 1* (6 ft. x 6 ft. x 6½ ft. inside measurements) with inside walls and

ceiling constructed of rigid insulating board, air temperatures were maintained at 89–92°F. and relative humidity at 60 to 70 per cent. *Room 2* was similarly constructed, with air temperatures and humidity kept at the same level as in room 1. However, it was lined throughout with aluminum foil glued to the wall board, and contained six cold plates (20 in. by 42 in.) which were installed on two opposite side walls and connected to a condensing unit located outside the enclosure. These plates were kept at 32 to 40°F. and acted as absorbing surfaces for radiant heat liberated within the room. They were exposed to the air and naturally imposed a heavy load on the air-conditioning equipment by their cooling and dehumidifying action. In both rooms, however, air conditions were satisfactorily maintained at the desired temperature and humidity levels, with not over 2°F. difference in air temperature at points one foot from the ceiling and one foot from the floor. *Room 3* (8 ft. x 8 ft. x 7 ft. double walled steel, with 1 in. rock wool insulation) was also lined with aluminum foil and equipped with an air cooling unit connected to an outside compressor. In addition it had arrangements for an input of radiant heat from ordinary coil reflector units (400 watt total capacity). With no heaters operating, the air temperature could be reduced to 32°F., and with all heating and cooling capacity in use, air temperatures stabilized at 70 to 76°F., varying slightly with outside temperatures. *Room 4* (similar in construction to room 3) was air conditioned for a 66 to 70°F. level by an ordinary room conditioning unit, with no radiant heat or cooling surface exposed within the room. In all four rooms adequate and similar ventilation was supplied by exhaust fans and proper air inlets, using inside laboratory air. Air mixing within the rooms was accomplished by recirculating units placed inside the respective rooms.

Experimental results. *Effect on human subjects.* Preliminary tests on human subjects indicated distinct discomfort in room 1, with free perspiration and a smothering sensation. In room 2, with identical air conditions, the stuffiness and smothering sensation were lacking, and adequate body cooling took place. In fact for a person to sit there working several hours, it became necessary to cover over part of the cold plates to prevent actual body chilling. One of us, in caring for the mice during that part of the study, found it necessary to wear a woolen sweater in this room even at 89 to 92°F. air temperature and 60 to 70 per cent relative humidity. In room 3, with the air at 32°F. and no radiant heat on, body chilling was severe; but delightful comfort was achieved within a few minutes after the heaters were turned on, even though air temperatures were still below 40°F. and the exhaled breath still visible. After all heater and cooler units had been in operation for days or weeks, this room was uncomfortably warm for human occupants within a half hour after entrance, at air temperatures of 70 to 76°F., no matter how lightly clothed. In room 4 sub-

jects were cool but comfortable when dressed in usual indoor winter clothing.

Effects on mice. Groups of healthy young white mice, which had been grown in the control room, were placed in the four test rooms. Every precaution was taken to have the four groups as similar as possible when placed in the rooms at three weeks of age. All were fed the same optimal diet as was used in previous work. Weekly weighings were made on each individual mouse to get the growth curve, and the age at onset of fertility was obtained by frequent matings with adults of known fertility as soon as the mating age was reached. Each group consisted of 10 males and 20 females.

In table 1 are given the results of these mouse observations. It is seen that fastest growth and earliest sexual development occurred in the group

TABLE 1

Growth and development of white mice under variations in ease of body heat loss

	ROOM 1 MOIST HEAT	ROOM 2 MOIST HEAT—RADIANT COOLING	ROOM 3 COLD AIR-RADIANT HEATING	ROOM 4 AIR COOLED
	grams	*grams*	*grams*	*grams*
Average weight in grams of males when placed in rooms at 3 weeks of age	11.1	10.8	10.8	10.5
Same at 10 weeks of age	26.1	29.3	27.4	28.3
Same at 15 weeks of age	28.3	32.0	29.4	30.2
Average age in days of females at delivery of first litter	63.9*	55.1	56.3	56.2
Average number per litter	6.5	7.7	7.6	7.7

*Half of the 20 females in this room have still shown no fertility at 16 weeks of age, even with repeated matings.

kept in room 2, these animals developing even more rapidly than did those in room 4 where best development had always previously prevailed. In room 1 growth and development were suppressed by the moist heat, just as had been found in previous studies. In room 3 a suppression of growth also took place, even though air temperatures were only 70 to 76°F. and relative humidity low. Good health was maintained by the animals of all groups, with not a single death during the four months of observation. Accompanying the growth stimulation in room 2 there was an earlier onset of fertility and the birth of litters about $\frac{1}{5}$ larger in numbers than in room 1 where growth suppression was accompanied by the birth of smaller litters of less viable young.

Discussion of results. Whether judged by human comfort or mouse growth standards, it is evident that in room 2 the depressing effects of moist warmth have been completely obviated by facilitating loss of body

heat by radiation. With air at 89 to 92°F. (practically summer dry skin temperature) and relative humidity of around 65 per cent, heat loss by conduction and convection can be only slight, and only by free perspiration and rapid air movement can evaporation be effective. The finding that body comfort and optimal animal growth can be secured in such a room, below the perspiration point, means that removal of practically all body heat through radiant channels is physiologically possible, and apparently quite desirable. The metabolic depression of room 1, quite typical of tropical moist heat conditions, can be completely overcome by facilitating radiant heat loss alone, without any change in air temperatures or humidity. Just why the air of room 2 should be so much more pleasant to breathe than that of room 1 is not at present explainable, since they are apparently identical. The effective factor may perhaps concern radiant heat loss from the nasal mucosa directly.

The suppression of growth of the mice in room 3 illustrates well the contention that it is the ease or difficulty of body heat loss which determines the metabolic or internal combustion level, and all the other vital functions thereto attached. No matter what the air temperature level, if the radiant heat load falling on the skin is so great as to make difficult the dissipation of this heat and that internally produced, then the body's response will be a reduction in internal combustion and a slowing down of its metabolism.

There now remains little doubt that general metabolic level is dominated by the ease with which body heat can be lost. The depressing effects of atmospheric moist heat can be completely overcome by properly facilitating body heat loss through radiant channels alone. This holds either in animal experiments or in human comfort. Likewise, the stimulation of cool surroundings can be turned to depression by increasing the radiant heat load on the organism. It thus becomes evident that the avenue of heat loss is unimportant,—it is the actual net rate of loss that serves to limit the general metabolic level of the individual.

These findings have two important bearings in the physiology of man and warm-blooded animals. First is this basic dominance exercised over general combustion level in the body. Second is the fact that a high metabolic level can be maintained by animals whose only avenue of body heat loss is through radiant means. Thus the usual partition of heat loss between conduction, convection and radiation channels is not a necessary physiologic condition.

The bearing of these findings on the problems of interior conditioning have been discussed elsewhere (3), in a paper presenting the physical aspects of the reflective radiant conditioning scheme here used. The foil wall coverings in rooms 2 and 3 act as passive reflectors of radiant heat in the rooms (95 per cent reflectivity), but maintain a surface temperature

always at that of the adjacent air. In room 2 the surface temperature of skin, clothing, wood, iron, etc., falls well below that of adjacent air, because of heat loss by radiation to the cold plates. Body heat loss is thus made easy, even though air temperatures be at or above that of the skin (91°F. in this room). In room 3 these same materials exhibit a temperature considerably above that of adjacent air.

The relationship we have demonstrated between ease of body heat loss and basic bodily functions has a direct bearing on many types of animal experimentation. It would seem important that certain standard environmental conditions be established for laboratory animals if the experimental results of one climate or season are to be compared with those of other lands and temperature conditions.

CONCLUSIONS

1. Such basic metabolic factors as growth, rate of development, and fertility are shown to be dependent on the ease with which body heat can be dissipated.

2. The avenue of heat loss seems unimportant. Adequate loss by radiation alone provides just as effective body stimulation as does a similar net rate of loss through the usual combined avenues of conduction, convection and radiation.

3. It would seem advisable that standards of environmental conditions be established for laboratory animals, so that results obtained in different climates and seasons can justly be compared.

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THE ABSENCE OF A SIGNIFICANT GLUCOSE-LACTIC ACID CYCLE (INVOLVING THE LIVER) IN NORMAL UNANESTHETIZED DOGS

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The work of Himwich, Koskoff and Nahum (1) supported by the observations of Cori (2) has led to the concept of a glucose-lactic acid cycle in which lactic acid liberated into the blood stream by muscular tissue is resynthesized into glucose (or glycogen) by the liver. The literature is in general agreement that under acute experimental conditions less lactic acid is found in hepatic than in portal blood (2, 3, 4, 5). In a few experiments on unanesthetized dogs, on the other hand, Doubinsky (6) found the liver adding lactic acid to the blood. No other observations on chronic, unanesthetized animals appear to have been reported. Because Tsai and Yi (7) and the authors (8) have shown that experiments involving anesthesia and surgery completely distort the normal processes of hepatic carbohydrate metabolism, the question of whether the glucose-lactic acid cycle occurs in normal, unanesthetized animals in the absence of excitement or exercise remains unanswered.

In our previous report (8) dealing with the effect of oral glucose on the sugar and lactic acid changes in the blood passing through the liver (normal, unanesthetized dogs) we adopted the general assumption that the liver under normal conditions removes lactic acid from the blood, although in none of the experiments of that series was such removal demonstrable by the method employed. While continuing similar experiments, involving the sampling of arterial, portal venous, hepatic venous and systemic venous bloods in unanesthetized animals by the angiostomy technique of London (9), we have had occasion to make many determinations of glucose and lactic acid in normal fasting dogs from which the removal or addition of these substances by the liver can be calculated. The results of these determinations do not support the concept that the glucose-lactic acid cycle is a significant factor in the carbohydrate metabolism of such animals. However, we wish to make it clear at this point that we do not doubt the existence of the glucose-lactic acid cycle under certain conditions. The data to be described cast doubt only upon the importance of the cycle in

the normal fasting animal in the absence of excitement or strenuous exercise.

METHOD. Dogs were provided with cannulae on the hepatic and portal veins by a two-stage operation according to the method of London (9). Our experience with this method for obtaining portal and hepatic blood samples without anesthesia is most satisfactory. Arterial blood samples were obtained by direct puncture of the femoral artery. The three samples were almost always obtained within three minutes. Determinations of "true" blood sugar and of blood lactic acid were made as previously described (8), the error of the blood sugar method being as then stated $\pm$ 1 mgm. per 100 cc. The error of the method for blood lactic acid has been determined by 24 analyses on 2 samples of blood, and the maximum variation found to be similarly $\pm$ 1 mgm. per 100 cc. Systemic venous blood samples were taken from either fore or hind leg veins without compression. Differences between arterial and systemic venous or arterial and portal venous samples were determined by subtraction. Differences between the outflowing and inflowing blood of the liver were determined by subtracting a weighted average of the portal and arterial values $\frac{(3 \times \text{portal} + \text{arterial})}{4}$ from the hepatic venous value. Although it is probable that the contributions of the hepatic artery and the portal vein to the total blood flow of the liver may vary considerably from a 1:3 ratio, substantially no error is introduced in these experiments by such an average because in the fasting animal the difference between arterial and portal venous values is relatively small. The differences were averaged and the standard errors (S.E.) of the average differences were determined by the usual statistical methods.

All samples were drawn after 18 hours' fasting. The dogs were trained until accustomed to the procedure, and every attempt was made to avoid excitement. When an animal was obviously excited no samples were taken until further daily training had made it possible to lift the dog on the table and draw the blood samples without apparent disturbance. The fact that in 44 experiments there were only 8 in which the blood lactic acid level was above 20 mgm. per 100 cc. indicates that we were successful in avoiding excitement. A total of 22 angiostomized but otherwise normal animals was used in these experiments.

RESULTS. The average differences, with their standard errors, of the lactic acid and glucose in arterial and systemic venous blood, outflowing and inflowing hepatic blood, and arterial and portal venous blood, are summarized in table 1. Our finding of an average retention of 4.8 mgm. of glucose per 100 cc. of blood by muscle (plus skin, bone, etc., i.e., arteriovenous difference) is in accordance with the results of previous investigators who have studied unanesthetized dogs (2). The differences are 7.8 times the standard error in the case of lactic acid and 6.5 times the standard

error for glucose, and are therefore almost certainly significant. For lactic acid differences between the blood leaving and the blood entering the liver the results are not significant, the average retention of 0.28 mgm. per 100 cc. being actually less than the standard error. This conclusion is substantiated by the fact that in 32 instances out of 44 the lactic acid difference was within the limits of error of the method, showing a definite retention only 7 times and definite output of lactic acid by the liver in 5 cases. This does not even indicate a trend toward retention. For glucose, on the other hand, the average hepatic output of 9.1 mgm. per 100 cc. is 13.4 times the standard error, and an output beyond the limits of error of the method occurs 44 times out of 47. It is apparent that in the

TABLE 1

Average differences (and standard errors of averages) between inflowing and outflowing hepatic blood, arterial and portal venous blood, and arterial and systemic venous blood for glucose and lactic acid

	NUMBER OF DETERMINATIONS	AVER. DIFF.	S. E. OF DIFF.	NUMBER OF DETERMINATIONS EXCEEDING ERROR OF METHOD INDICATING	
				Retention	Output
		mgm.			
Arterial minus systemic venous:					
Lactic acid	16	−3.1	∓0.40	0	10
Glucose	16	4.8	∓0.74	13	0
Hepatic outflowing minus inflowing:					
Lactic acid	44	−0 28	∓0.41	7	5
Glucose	47	9.1	∓0.68	0	44
Arterial minus portal venous:					
Lactic acid	44	−0.68	∓0.51	5	10
Glucose	47	2.9	∓0.33	22	1

fasting animal the average retention of lactic acid which could escape detection can hardly exceed 1.1 mgm. per 100 cc. (average difference plus twice S. E.); the actual glucose output varied from 2 to 23 mgm. per 100 cc., 58 per cent of all the values lying between 6 and 13 mgm. The average output of lactic acid by the gastrointestinal tract (arterio-portal difference) is 0.68 mgm. per 100 cc. which but slightly exceeds the standard error and is not significant, although there are 10 determinations in which the apparent output exceeds the error of the method and but 5 in which there is retention beyond the limit of error. Glucose retention by the gastrointestinal tract, while less than that of leg tissue, seems to be established since the average retention of 2.9 mgm. per 100 cc. is 8.8 times the standard error.

In table 2 the data for 3 successive lactic acid and glucose analyses on

arterial, portal, and hepatic samples in 3 dogs are given. These data are not included in table 1, but are presented to show that successive samplings do not materially alter the values obtained.

Discussion. The observation that the resting fasted animal does not show lactic acid retention by the liver is not in accordance with our preconceived ideas, for we had assumed that the glucose-lactic acid cycle as generally understood was operative in such animals. The absence of lactic acid retention by the liver in normal unfed dogs, even though in such animals the muscle masses (as represented by leg tissue) are liberating lactic acid into the blood stream, need not, however, be considered incompatible with previous evidence. It is known that many tissues other than the liver, especially heart and brain, are capable of utilizing lactic acid

TABLE 2

Successive blood glucose and lactic acid determinations on hepatic and portal venous and arterial bloods of normal dogs to show constancy of results

	TIME	GLUCOSE			LACTIC ACID		
		Hep.	Por.	Art.	Hep.	Por.	Art.
	minutes						
Dog I	0	91	80	84	15	12	14
	20	89	81	83	14	10	14
	60	92	82	84	12	11	10
Dog II	0	93	91	90	13	12	10
	20	89	87	87	10	8	6
	60	92	88	91	9	8	8
Dog III	0	83	70	69	11	11	8
	20	76	65	66	15	14	11
	60	74	65	66	8	11	11

from the blood stream. In fact reconversion of lactic acid to glucose by the liver might appear to be an uneconomic process when we consider that utilization by other tissues apparently need not involve such reconversion. Aubel (10) has shown that lactic acid liberated into the blood by prolonged muscular activity can disappear in animals in which the liver is excluded from the circulation and the blood sugar level is maintained artificially. Bollman and Mann (11) believe that the increase in blood lactic acid after hepatectomy is secondary to the surgical procedure, since the level returns toward normal and the liverless animal can dispose of large quantities of injected lactic acid. Hahn (12) has shown that liver injury does not delay the removal of lactic acid from the blood.

Himwich *et al.* (1) report a considerable hepatic retention of lactic acid in normal dogs under amytal anesthesia, or decerebrated; the blood lactic

acid levels in their animals were well above normal, being from 34 to 78 mgm. per 100 cc. in the instances reported. Wierzuchowski and Sekuracki (3) have also reported retention of lactic acid by the liver in acute experiments (amytal anesthesia) as have Giragossintz and Olmsted (4). McClure (5) in dogs under morphine and urethane notes that the pH of hepatic blood is higher than that of portal or arterial, and suggests that the difference may result from retention of lactic acid. Doubinsky (6), as previously mentioned, is the only investigator to study unanesthetized animals; his data on a small series indicate production of lactic acid by the liver. We believe that the data obtained in animals under acute experimental conditions cannot be taken as indicative of the metabolic processes that occur in normal unanesthetized animals. Tsai and Yi (7) and we ourselves (8) have shown in another connection that acute experimental conditions produce marked changes in the behavior of the liver, and Gesell, Krueger, Gorham, and Bernthal (13) have shown that in acute experiments under amytal the blood lactic acid is likely to be high, although amytal itself does not increase the lactic acid (14). The results of Doubinsky may be explained by the small series employed; we have also noted an output of lactic acid by the liver in some animals.

Our data in no way invalidate the observations of others that conversion of lactic acid to glucose occurs in the liver after epinephrine, exercise, or any other condition which causes the blood lactic acid level to rise. Hepatic retention of lactic acid, in our opinion, is an important factor in maintaining the acid-base balance and preventing an excessive increase in lactic acid under such conditions. We believe, however, that conclusions concerning normal metabolic processes cannot be drawn from acute experiments involving anesthesia and surgical procedures. In a number of acute experiments (nembutal anesthesia) we have confirmed the observation of others that the liver uniformly removes lactic acid under these conditions.

It is interesting to calculate from our data the presumable output of glucose by the liver per 24 hours. Such calculations cannot be taken as more than suggestive, because of the number of variables involved, but may be assumed to represent a figure not far removed from the average. Blalock and Mason (15) have recently redetermined the average blood flow through the liver in unanesthetized dogs and their data agree well with those of previous investigators. According to their average of 27 cc. of blood flowing through the liver per kilo body weight per minute, 777.6 liters should pass through the liver of a 20 kilo dog daily. The majority of our dogs weighed approximately 20 kilos. Using our average glucose output of 9.1 mgm. per 100 cc. we find that the liver should supply 70.7 grams of glucose per day or 0.15 gram per kilo per hour. It is interesting to note that this figure is not too far from the glucose requirement of the

hepatectomized animal as determined by Mann and Magath (16) (0.25 gm. per kilo per hour). The difference could easily be made up by glucose absorbed from the gastrointestinal tract and not retained by the liver. In contrast the possible glucose formation from lactic acid retained in the liver but not detected by our methods could amount to only 8.6 grams per day.

Comment on our data for arterio-portal differences is hardly necessary. A utilization of approximately 3 mgm. of glucose per 100 cc. of blood or about $\frac{3}{5}$ of the amount utilized by resting leg tissues seems entirely reasonable. While statistical analysis of our data does not support the conclusion that the gastrointestinal tract tissues add lactic acid to the blood stream, it seems not improbable that the addition of small amounts does occur.

SUMMARY

The amounts of glucose and lactic acid added to or withdrawn from the blood passing through the gastrointestinal tract, liver, and leg tissue in fasting, unanesthetized dogs were determined by analyses of the blood entering and leaving these organs. Blood samples were obtained by the angiostomy method.

In 16 experiments, the average retention (per 100 cc. of blood) of glucose by leg tissue was 4.8 $\mp$ 0.74 mgm., and output of lactic acid was 3.1 $\mp$ 0.40 mgm. Output of glucose by the liver averaged 9.1 $\mp$ 0.68 mgm. in 47 experiments. The average retention of lactic acid by the liver was 0.28 mgm. with a standard error of $\mp$ 0.41 in 44 experiments. The gastrointestinal tract removed an average of 2.9 $\mp$ 0.33 mgm. of glucose per 100 cc. of blood; no significant addition of lactic acid was demonstrable.

These results were interpreted as showing that a glucose-lactic acid cycle in which the liver removes lactic acid from the blood stream and converts it into glucose or glycogen does not occur to a significant extent in normal unanesthetized dogs, in the absence of excitement or vigorous exercise. Previous experimental data suggesting the contrary have been obtained under conditions which alter the normal processes of carbohydrate metabolism in the liver. There is, therefore, no conflict between previous evidence and these observations. The authors do not question the concept that removal of lactic acid from the blood by the liver may be important in regulating the acid-base balance, and occurs after epinephrine or strenuous exercise.

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AN EXPERIMENTAL STUDY OF THE STANDING WAVES IN THE PULSE PROPAGATED THROUGH THE AORTA[1]

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With his development of adequate manometers, Otto Frank (6) furnished the first conclusive evidence for discrepancies in pulse form and pulse pressure in different portions of the arterial tree. He showed that the pulse pressure recorded from a dog's femoral artery might greatly exceed that taken simultaneously in the subclavian; and that the form of the pulse at the femoral resembled closely that of a pulse in a central artery recorded with a low-frequency manometer. The introduction, by Hamilton, Brewer, and Brotman (10), of the metal membrane manometer with a lens mirror facilitated the extension of such researches to previously inaccessible arteries and to human subjects. Hamilton, Woodbury, and Harper (11) have published simultaneous pressure records from a human, showing the systolic pressure higher in the femoral than in the axillary artery and even higher in the dorsalis pedis than in the femoral. The present experiments were designed to show the development of such changes through the hitherto unexplored regions of the aorta, and to demonstrate the nature of the transformations that occur.

METHODS. Although it is hoped that these experiments may have a bearing on human physiology, their nature precludes the use of human subjects. All the experiments reported here were done on dogs without opening chest or abdomen or having to resort to artificial respiration. The records themselves are evidence of the healthy condition of the circulation and the absence of shock. Each dog received morphine sulfate subcutaneously, about 8 mgm. per kgm. In the earlier experiments this was the only anesthesia necessary; later, when the subclavian artery was exposed or the aorta was occluded, ether was used in addition.

The manometers employed were of the type previously described by Hamilton, with coin silver membranes (beryllium-copper alloy was used in one recent experiment). Their frequency, when coupled to the can-

[1] Preliminary reports of this study were made before the American Physiological Society at the meetings in Washington, Memphis, and Baltimore: see This Journal **116:** 36, 1936; **119:** 297, 1937; **123:** 54, 1938.

nulae, usually varied from 100 to 150 double vibrations per second. Records were made on 6 cm. electrocardiograph paper at a distance of about 2 m.

The cannulae were made by soldering 12 to 31 cm. lengths of 14 to 18 gauge needle tubing into standard fittings for attachment to the leaden tubes. They were marked off in centimeters, their ends were dulled with beads of solder, and they were filled with 8 per cent sodium citrate as anticoagulant. Such cannulae were inserted into the femoral, carotid, and left subclavian arteries. By moving them in and out and taking records at various measured distances, pairs of simultaneous pressure pulses could be obtained from practically any parts of the carotid-aorta-iliac-femoral system. In some dogs the arch of the aorta could be passed from above by a long cannula down the right carotid; in others only through the left subclavian.

The records were measured under the low power of a compound microscope (55 mm. objectives, 4×). Our first method, using an ocular micrometer for time and a Vernier-reading mechanical stage for pressure measurements, was replaced by a simpler and easier one employing a measuring plate. This plate, made on a lantern slide, was a contact print of a 10:1 photographic reduction of millimeter paper.[2] With the optical magnification and camera speeds used, estimations of time intervals were reproducible to about 0.001 sec., and of pressure to about 0.5 mm. Hg.

Results. The upper portion of figure 1 shows accurate reconstructions, all to the same time and pressure scales, of pressure pulses from successive points, as indicated, out the thoracic and the abdominal aorta and into the iliac artery. Each is compared with the pulse recorded simultaneously from the arch of the aorta.

The noteworthy points of comparison between these curves can be gleaned more easily from the lower portion of the figure, where several of them are superimposed. In spite of care in selecting pulse cycles with similar control curves, there was unavoidable variation in systolic and diastolic pressures and duration of systole; consequently some of the original curves have been shifted slightly to make them fit into the composite picture which is therefore semi-diagrammatic. This figure shows the following transformations taking place in the pulse as it progresses down the aorta:

1. The transmission time of the start of the wave, as would be expected, is progressively longer. A more detailed analysis of this feature will appear in a subsequent paper.

2. The diastolic pressures at the arch and at the various peripheral points are essentially the same. Otto Frank's original records showed the diastolic pressure in the femoral considerably lower than that in the

[2] The negative from which our plates were made is the property of the Tommins Studio, Augusta. Additional plates can be printed for a reasonable charge.

carotid or subclavian; but careful measurements of many long series have convinced us that such differences, if any, are scarcely appreciable at the end of diastole when all the various waves have died down. This conclusion is eminently reasonable, since the slight resistance to flow in

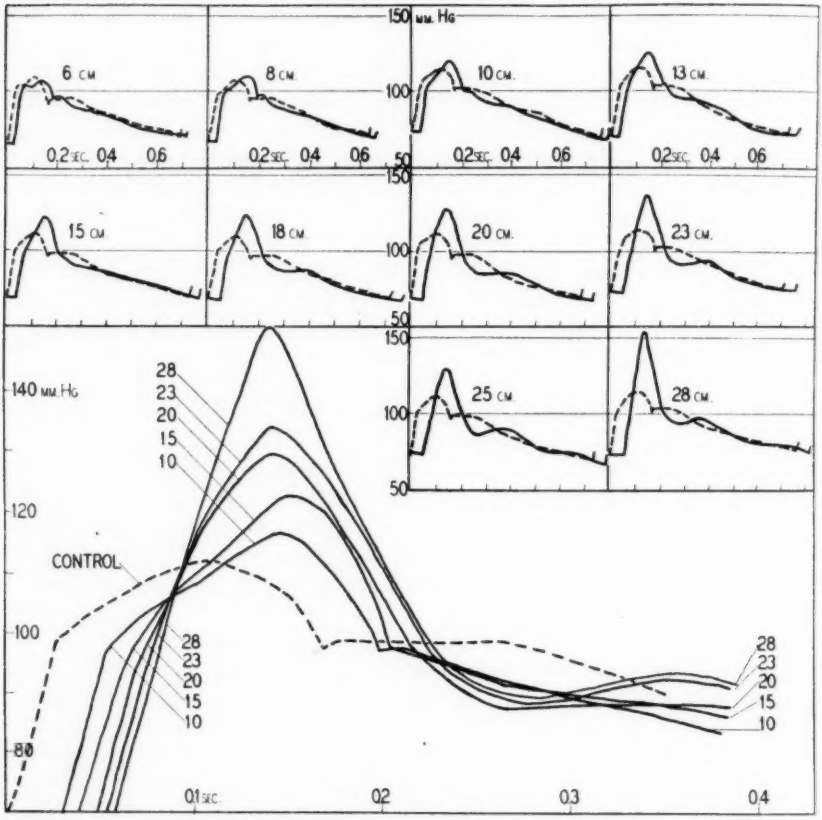

Fig. 1. Reconstructions of aortic pressure pulses, showing comparison between control in aortic arch (dotted lines) and records taken simultaneously with their controls at indicated distances down the aorta from the arch (solid lines). Below, five of the above ten, semi-diagrammatically superimposed on a somewhat larger scale, with a representative control.

the large central arteries and the relatively low blood velocity at the end of diastole do not imply any considerable pressure head.

3. Starting somewhere in the thoracic aorta, the systolic pressure becomes progressively higher. At its maximum in this experiment the pulse pressure exceeds that at the arch by more than 90 per cent.

4. As the wave travels down the aorta the slope of the first rise remains quite constant. The anacrotic shoulder gradually disappears until the initial upstroke becomes a smooth rise to the systolic peak (which at that point appears to have reached its maximum height).

5. Although the start of the wave has an increasing transmission time, this is not true of the peak. Beyond the first recorded position the systolic peak is essentially stationary—simultaneous at all points. The slight variations in its time show no consistent trend in either direction.

6. The incisura, prominent at first, is gradually lost. Not at the same point in the cycle but somewhat later, the diastolic dip characteristic of the femoral pulse develops, and beyond it a rise. For many reasons it is difficult to judge exactly what point to call the bottom of the diastolic dip or the top of the succeeding rise. This is chiefly because a smooth curve of pressure fall during a quiet diastole, upon which the waves are superimposed, is not horizontal; nor is it even an incline of constant slope. Moreover, the waves are relatively long and without sharp inflections. As nearly as can be estimated, however, these dips and rises show no consistent trend either forward or backward in time and appear to be as stationary as the systolic peak.

7. It is noteworthy that the control curve of pressure at the aortic arch does not fall abruptly after the incisura but goes through a plateau phase first. The maximum height of this plateau above the expected simple decline of pressure occurs at about the same time as the bottom of the diastolic dip of the more peripheral pulse.

8. In addition to these observable characteristics of the curves, the areas under them have been carefully measured with a planimeter. Despite the great variation in pulse form and systolic pressure, the areas under each pair of curves (and therefore the mean pressures of these pulses) are identical within the limits of error.

The results of a second experiment, similarly reconstructed and analyzed but not pictured here, showed again a stationary systolic peak but with certain interesting differences. The dog was much smaller, with a diastolic pressure of about 135 mm. Hg instead of 70 mm. and with a well filled pulse which continued to rise almost throughout systole. There was little difference in heart rate, but systole was a little longer. In this experiment the stationary systolic peak developed earlier, preceding rather than following the peak at the root of the aorta. In this small dog we were able to reach with our cannula to a point where the two peaks were superimposed. When the cannula was withdrawn only a short distance, there was an abrupt change to the curve with the early peak.

The fact that the systolic peak occurs at the same time through each of the series just described suggests that a system of standing waves is involved. Putting aside for a later discussion the nature or cause of such

standing waves, it can be said that from a descriptive standpoint they should satisfy three requirements: 1, since pressure can be maintained and varied at either end of the tube, two divisions of the system should be distinguishable whose pressure waves are mutually reciprocal, 180° out of phase; 2, in each division all parts should be in phase, should experience rises and falls of pressure simultaneously, though the amplitude may vary; and 3, between the two divisions there should be a node or point of zero pressure fluctuation.

The simultaneous peaks and dips described above seem to satisfy the second of these requirements; and the apparent reciprocity of the diastolic dip with the plateau of the control curve suggests that the first requirement is also met. However, the mapping of the upper half of the system is not complete and the node is defined very poorly. In order to demonstrate all three phenomena clearly it was necessary to isolate parts of the system and accentuate the waves, then explore the isolated sections more thoroughly than before. To this end the experimental procedure was modified as follows.

Around the tip of the cannula which entered the right femoral artery was tied a small balloon of thin rubber. Through a section of 26 gauge needle tubing smoothly soldered along the cannula, the balloon was connected to the compressed air line so that it could be inflated at will, occluding the aorta wherever it was placed. Another long cannula was passed down the right carotid, over the arch, and down the descending aorta, so that when both cannulae were inserted to their hilts their tips overlapped for several centimeters (see sketch in fig. 3). Effective occlusion and release were confirmed by recording the pulse through a cannula tied into a small artery at the left knee.

The tip of the occluding cannula was fixed at a known position in the lower part of the aorta. The upper cannula was pushed in until the two tips were almost touching, and a continuous record was taken of pulses before, during, and after occlusion. The upper cannula was then pulled back 2.5 cm. and a similar record was taken. This was repeated at 2.5 cm. intervals until the whole segment had been explored back over the arch. Such series of records were taken for each of several locations of the occluding cannula.

The results of one series are reproduced in figure 2. In the upper half of the picture is one complete group of cycles before, during, and after occlusion. The lower part shows one cycle from the occluded portion of each of the subsequent groups of the same series. Throughout the series illustrated, the femoral cannula was fixed with its recording tip and occluding balloon about 2 cm. above the diaphragm. The proportion of the normal drainage cut off by occlusion here (indicated on the record

by the disappearance of pulsations at the knee) is shown by the large increase in diastolic pressure and pulse pressure above this point.

Although this dog's circulatory system remained in good shape throughout the long experiment and showed no evidence of shock, repeated occlusions modified the normal conditions sufficiently so that the usual waves in the unoccluded pulses were not prominent enough to be studied. However, the reproducibility of conditions during occlusion is attested by the constancy of the pulse form recorded by the occluding cannula in all records.

In contrast to this constancy at the point of occlusion, examination of the curves brings out the following changes in the form of the pulse

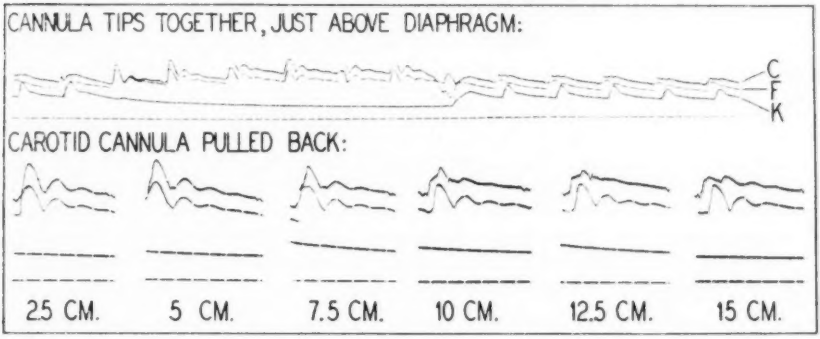

Fig. 2. Mapping of the waves in thoracic aorta above an occlusion (see text). Photographs of original records, without calibrations for actual pressure values. Time intervals $_1{}^1{}_2$ sec. Top line on each record (*C*), cannula down from right carotid; middle line (*F*), cannula up from right femoral; bottom line (*K*), control cannula at left knee to confirm effective occlusion and release; below, common base line.

recorded from points successively nearer to the heart in the isolated aortic segment.

1. The wave, as would be expected, starts earlier.

2. A break develops in the anacrotic limb, which an accurate analysis of transmission times shows to be due to reflection at the point of occlusion.

3. The early diastolic waves do not show such a shift, but remain in phase with those at the occlusion until a point 12.5 cm. away is reached. Here they disappear, but 2.5 cm. farther back, at the top of the arch, they reappear exactly opposite in phase to those farther down. A node is thus clearly shown, a point of minimum pressure fluctuation with the waves on either side 180° out of phase or mutually reciprocal.

By fixing the point of occlusion at several different places in the aorta,

the node was shown to shift according to the length of the isolated segment. This is summarized in the diagrammatic sketch of figure 3, where the location of the node associated with each occlusion is indicated.

The smooth curves of fall of pressure beyond the occlusion in this and many similar experiments are being measured. Results thus far indicate that careful analysis of them will produce, for early publication, interesting data on the relationships between elasticity and outflow in the arterial reservoir.

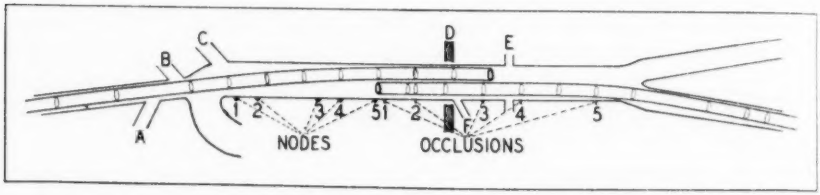

Fig. 3. Diagrammatic sketch of system studied, showing range of operation of two cannulae and points at which series of records were taken (indicated by round marks on the cannulae). The occluding balloon at the tip of the femoral cannula is not shown. With occlusion of the lower aorta at the five indicated positions, reversal of phase of the early diastolic waves was observed at the correspondingly numbered nodal points in the upper aorta. Landmarks indicated as follows: *A*, right subclavian artery; *B*, left common carotid; *C*, left subclavian—used as the upper entrance in later experiments; *D*, diaphragm; *E*, renal arteries; *F*, coeliac axis.

Discussion. In attempts to explain the transformations undergone by the arterial pulse, four principal lines of emphasis have arisen. Bramwell (5), illustrating his theory with experiments on mercury in rubber tubes, pointed out that the discrepancy in velocity between the start and the peak of the pulse wave may result in a change of form. Since our results on intact animals show the slope of the first upstroke of the pulse wave to remain essentially the same, at least from thorax to femoral, and the peak to be stationary, we believe that Bramwell's interesting phenomena should be regarded as playing a negligible part in the argument.

Many workers have spoken of the fusion of reflected waves with the outgoing pulse, without going further into a consideration of the periodicity and simultaneity of the pressure variations so produced.

Gladstone (8) and formerly Bazett (1) have emphasized the rapid movement of the column of blood and have stated that some such phenomenon as that called water-hammer must be involved: either that the kinetic energy of the blood is converted into lateral pressure as it slows in the widening bed (Pitot or Bernoulli effect); or that the momentum of the column, on meeting resistance to flow, carries it on to distend the arteries in a surge (true water-hammer).

In the course of his studies on the adequacy of recording manometers, Otto Frank (6) early recognized the resemblance between the femoral pulse and a low-frequency recording of the central pulse, and thus developed the concept of the natural vibration period of the central arterial reservoir as expressed in the peak-to-rise time of the pulse at the femoral.

Certain anatomical and mechanical facts have been pretty definitely established. Since the arteries are distensible their volume is intermittently increased by the periodic emptying of the left ventricle into them. Since they are elastic this distention is accompanied by an increase in internal pressure; and during diastole they return to their original volume by expressing the increment through a resistance which Whittaker and Winton (14) have shown to be independent of the pressure. Their elasticity accounts too for the propagation in them of waves set up by the sudden filling at the root of the aorta. The walls are not homogeneous and may not be isotropic; their elastic characteristics, therefore, as Wilens (16) has pointed out, are not amenable to analysis by simple physical methods. The volume-elasticity coefficient is known to differ throughout the system and to vary with the internal pressure. Where arteries divide, in general each branch is smaller than the parent tube but the total cross sectional area is greater.

Grashey (9) enumerated the changes in an elastic tube which produce reflection of a wave with its phase unchanged. They are the same as those which would increase the velocity of the wave or the resistance to flow of the liquid in the tube: namely, a constriction of the tube (with an obstruction or a closed end as the limiting case) or an increase in its elasticity. Similarly, a wave is reflected with its phase reversed when the tube suddenly widens (an open end is the limiting case) or its elasticity decreases: either of these conditions would both slow the wave and reduce the resistance to fluid movement.

If then wave reflection and resistance to flow go hand in hand, the pressure relationships reported here demonstrate that the three arguments outlined above diverge merely in their emphasis on different phases of the same phenomenon. Any increase in pressure within an artery is necessarily bound up with an increase in its content of blood. Looked at from this point of view, the standing waves demonstrated in our experiments show that the proximal and distal ends of the aorta-femoral system accommodate alternately, each at the expense of the other, an excess of blood. In a system without a continuous flow this would mean the oscillation of a certain amount of blood back and forth from one half of the system to the other. Actually, in a flowing stream, it may represent only an alternate acceleration and retardation of the flow from the proximal to the distal end.

Such a periodic sloshing, like that of water in a deep trough, can be

considered equally well from a physical viewpoint as a moving fluid meeting an elastic resistance or as the result of the resonance of reflected waves of suitable frequency. According to Grashey's principles the conditions producing the two phenomena are the same. In a manometer where the elastic resistance resides in a distensible membrane over the end of a rigid tube, Frank's original analysis (on the basis of the moving liquid) is adequate. Where the walls themselves stretch with a variable elasticity such treatment is far too cumbersome and a wave analysis is more profitable, as Frank himself pointed out. This is a reasonable method as well, since the actual energy transfer in such a continuously elastic system is through the medium of wave motion.

We believe, therefore, that in the light of the results reported here the concepts of reflected waves, standing waves, natural vibration period of an elastic system, and a sort of low-frequency water-hammer present no fundamental differences of theory that cannot be reconciled on a sound physical basis.

This argument follows the principles laid down by Otto Frank and clearly advocated in Maltby and Wiggers' (12) study of the pulse in an occluded artery. In view, however, of some recent criticisms, we would reiterate the point so thoughtfully discussed by them (12) that water-hammer and Pitot effect, though often confused, are two quite different phenomena.

Böger and Wezler (4) have taken exception to the published results of Hamilton, Woodbury, and Harper (11), suggesting that the upstream insertion of needles, together with an expected difference between lateral and end pressures, undoubtedly would account for the large discrepancies found between the systolic pressures in central and peripheral vessels. From fellow workers in other laboratories have come friendly inquiries upon the same point since the presentation of these results before the American Physiological Society. In support of our contention that such a factor cannot play an appreciable rôle in our results we urge the following considerations:

1. Analyzed on this basis such pressure differentials as were published by Hamilton, Woodbury, and Harper and such as appear here would correspond to velocities enormously greater than any yet measured or even thought of in these vessels.

2. It must be remembered that the whole series of unoccluded aortic curves here presented were recorded from a cannula pointing upstream, the only variable being the location of its tip. Such a cannula should at all times pick up the end pressure—the sum of the lateral pressure and the energy due to the velocity of the blood.

3. The planimeter determinations show that there is no addition to the measured energy or subtraction from it.

4. We have repeated the systolic augmentation experiment using a cannula with a plugged end and with openings around the side just behind the plug. The results obtained were practically identical with those in the earlier experiment.

The logical extension of the occlusion experiments reported here would be to find the anatomical end of the oscillating system by locating the point where occlusion made no difference in the position of the node or in the frequency of the standing waves. Most of our records suggest, however, that this point is not in the aorta, where it could be reached by our method; nor even in the iliacs, as Wezler and Böger assume (13). Records taken simultaneously at the knee and in the lower aorta show little or no difference in the time of the systolic peak. This we take as evidence that, at least in dogs, arteries even as far out as the knee are a part of the compression chamber whose natural period of vibration appears in the standing waves.

Theoretical considerations, together with careful comparisons of many series of records, lead us to believe that the natural point of reflection occurs where the transmission time of the foot of the wave (from the aortic valves) and the time from the foot to the stationary systolic peak are equal. Extrapolation of transmission time curves places this somewhere near the knee—or at equivalent distances along other pathways. When we consider the available knowledge about resistance to flow in large arteries and their branches, as well as the pulse velocity curves reported in a companion paper to this, it is our impression that such massive effects as the changes in form and pressure reported here cannot possibly result from reflections and resistances in distributing arteries; on the contrary, it seems more likely that they represent the summation of effects at many sub-terminal branchings at approximately the same distance from the main arteries of the thigh—distances, that is, which would be traveled by the wave in the same length of time.

Wezler and Böger's (13) location of the end of the central compression chamber in the iliacs resulted from a disregard of pressure relationships, an unsound application of the principles of wave reflection, and an irrelevant quotation of Gauer's (7) results.

The direct pressure records taken simultaneously from the axillary, femoral, and dorsalis pedis arteries of a human subject by Hamilton, Woodbury, and Harper (11), show the peaks and dips of the dorsalis pedis pulse lagging somewhat behind those of the femoral, suggesting that the two are not parts of the same vibration system. Bazett, Cotton, Laplace, and Scott (3), however, have already pointed out the absurdity of neglecting, in cardiac output studies, the many arteries which are filled during systole even though they do not seem to be a part of the central vibration system.

Finally we would point out that if there is obtainable some criterion for the start of the pressure pulse at the root of the aorta, the time from this to the standing peak at the femoral is a more nearly constant and more precisely measurable characteristic of the natural period of the elastic central reservoir than is the time from the systolic peak to the diastolic rise.

SUMMARY

1. A technique is described for recording, directly and adequately, simultaneous pressure pulses from different parts of the open or occluded system comprising the aorta and the iliac and femoral arteries of intact dogs.

2. Series of records taken by this method present the following findings:

a. The pulse pressure increases gradually from the aortic arch to the femoral, although the mean pressure remains constant.

b. The augmented systolic peak is not simply a propagated wave but is stationary. Beyond a nodal point somewhere in the thoracic aorta it occurs simultaneously at all points in the system. The time from the start of the wave at the root of the aorta to the time of this stationary peak is a characteristic function of the length and the elastic properties of the central arteries, probably as far out as the knee.

c. Along with the formation of this stationary peak, and following it in the pulse cycle, there develop alternate falls and rises of pressure which reciprocate with simultaneous rises and falls at the root of the aorta.

d. By moving an occlusion down the aorta, the nodal point between these reciprocating divisions of the system can be shifted in the same direction.

e. The transformations in form and pressure undergone by the pulse in its travel toward the periphery are thus experimentally shown and their cause is identified. By reflection of the propagated wave, with changes in the volume-elasticity properties of the vessels through which it goes, certain components of it resonate and the standing waves so produced are superimposed upon the fundamental pulse form.

3. It is shown that in the light of these experiments the application of sound physical principles permits a reconciliation of many controversial lines of emphasis in this field.

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AN EXPERIMENTAL STUDY OF THE VELOCITY OF THE PULSE WAVE PROPAGATED THROUGH THE AORTA[1]

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Although all parts of the arterial reservoir experience their maximum filling almost simultaneously and before the closure of the aortic valves, the front of the wave of filling proceeds with a finite velocity from the heart toward the periphery. Grunmach (5) in 1879 noted the possession of this knowledge by Erasistratus and Galen and its rediscovery by Weitbrecht in 1734. Since the classical studies of the brothers Weber, the transmission velocity of the arterial pulse wave has furnished the physically minded physiologist and the physicist of biological interests a most fascinating field of study. Moens (7) in 1878 was the first to state clearly and accurately the relationships between the physical factors which determine the velocity of propagation of a pulse in an elastic tube filled with a liquid: namely, the elasticity and thickness of the wall, the bore of the tube, and the density of the fluid. Bramwell and Hill (2) in 1922 altered the Moens equation to a more usable formulation, emphasized the variation of pulse velocity with intra-arterial pressure, and gave renewed importance to Roy's (9) fundamental determinations of the elasticity of arterial walls. Bazett and Dreyer (1) in the same year demonstrated clearly that the pulse velocity in peripheral arteries was greater than that in more central ones. A. H. Garrod (3), in 1874, had stated such a proposition, on the basis of experiments which did not clearly prove it, and confessed himself at a loss to explain the phenomenon.

Based upon the principles developed by these and many other workers, there has arisen a great mass of literature in which the velocity of the pulse wave is used as the key to a better understanding of pathological changes in the circulation, particularly those due to senility and hypertension. Direct pressure pulse records from experimental animals and less accurate surface sphygmograms from human subjects have given fairly consistent figures for the average velocity from the heart to the femoral artery. Gauer (4) and some others have reported surface sphygmograms repre-

[1] A preliminary report of this study was made before the American Physiological Society at the meeting in Baltimore, March, 1938: see This Journal **123:** 54, 1938.

senting the pulse in the upper abdominal aorta. However, the comparative inaccessibility of the aorta itself in intact animals and humans has heretofore precluded any detailed researches on the velocity at points between the heart and the iliac arteries.

The apparatus and methods described in the previous paper (6) and the records obtained in a lengthy study of the pulse form in the aorta offered a golden opportunity for obtaining accurate knowledge of the changes, if any, in the velocity of the pulse wave during its progress from the heart out to the femoral. All of the results reported here were derived from the analysis of direct records of pressure pulses taken with long cannulae which were run up the aorta from the femoral or down from the left subclavian

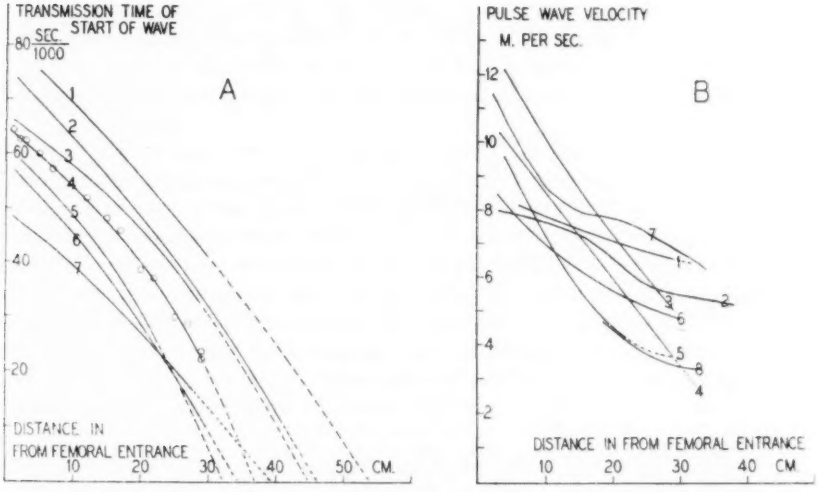

Fig. 1. Transmission velocity of the pulse wave in different portions of the aorta

artery, always combined with a control cannula pushed down a carotid to the aortic arch.

Results. Figure 1-A shows transmission time curves for seven dogs. The distance up the aorta was recorded from the cannula calibration, and the transmission time of the start of the pulse wave from the aortic arch to each cannula position was measured as previously described, to the nearest thousandth of a second. Several cycles at each point were measured, the averages were plotted, and smooth curves were drawn by inspection and extrapolated (dotted lines) to zero transmission time. To avoid confusion the plotted points were included for only one curve; the fit was about the same for all. From eleven to twenty points were used to de-

termine each curve. Each point represented the average of three pulses on curve 5, six to twenty pulses on the others.

From one animal to another there were variations in weight, length, age, and diastolic pressure: hence the differences in absolute transmission times and the extrapolated total aortic lengths. However, the curves are all similar in shape, concave toward the axes. On any one of them, the slope to the time axis at each point represents the velocity of the foot of the pulse wave at the corresponding point in the artery.

These velocities were measured on all seven curves at 1 cm. intervals by means of a Richards-Roope tangent meter, and the smoothed curves through the points thus determined, numbered to correspond with those in figure 1-A, were drawn to give figure 1-B. There is considerable variation between the experimental animals, but in all cases the pulse wave accelerates as it passes out to the femoral from the farthest point reached in the thorax. In most of the curves this acceleration is fairly steady, without any sudden breaks to which can be assigned an anatomical significance.

One possible exception should be noted, an observation which may or may not assume statistical significance with the analysis of a large number of records. Some of the transmission time curves showed a tendency to dip in one region, and at autopsy that cannula position seemed to be near the coeliac axis and the diaphragm. It is conceivable that the wave might accelerate slightly under the diaphragm, slow somewhat opposite such large drains as the coeliac axis and the renal arteries, and speed up again in the narrower vessel beyond. Our records so far suggest this as a possibility but are not yet sufficiently detailed or numerous to make the finding statistically significant. It has, therefore, been neglected in drawing the curves.

Included in figure 1-B is a curve (no. 8) calculated from a different type of experiment. Successive 1 cm. rings, from the heart down into the abdomen, were cut from the aorta of a dog freshly killed by hemorrhage and pneumothorax without ether. These rings were stretched between steel pins on a Scott tester made available to the Department of Microscopic Anatomy through an American Medical Association grant. With this machine the traction on each ring was continuously increased from 2.5 grams to 250 grams in one minute, and a continuous record of the corresponding elongations was drawn. The measured lengths and tensions were converted into volumes and internal pressures, the results were plotted, tangents were determined, and the pulse wave velocities were calculated according to the Bramwell and Hill transformation of the Moens formula. Although this series is not complete and there are no actually measured pulse velocities for this dog, the predicted acceleration is shown to be consistent with the findings from other direct determinations.

The next project was to study the variation of pulse wave velocity with intra-arterial pressure in different parts of the aorta. The recording tip of one cannula was placed in the aortic arch, another about at the diaphragm, and a third at the bifurcation into the iliacs. Cardiac arrhythmia furnished a wide range of normal diastolic pressures, which was extended upward by the injection of 0.5 cc. of 1:1000 epinephrine and downward by electrical stimulation of the intact right vagus.

The results of this experiment are shown in figure 2, where the velocity of each pulse wave is plotted against the diastolic pressure at which it was propagated. In the thoracic aorta normal variations and low pressures make little difference in the velocity. In the abdominal aorta there are

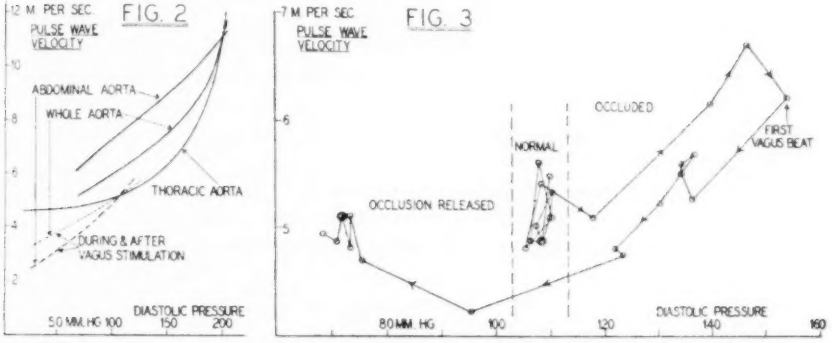

Fig. 2. Velocity of the foot of the pulse wave as a function of the diastolic pressure in two segments of the aorta. Discussion in text. The figure is too compact for inclusion of the 450 plotted points used to determine the curves.

Fig. 3. The effect of reflex depressor activity (in addition to that due merely to lowering of the pressure) upon the relationship between diastolic pressure and pulse wave velocity in an occluded thoracic aorta. Discussion in text. Time sequence of cycles indicated by arrows.

higher velocities and a steeper variation with pressure over the normal range of diastolic pressures. The curve for the whole length from arch to bifurcation, though plotted independently, is of course the resultant of the other two.

In plotting the curves for the whole aorta and for the abdominal segment an unexpected difficulty was encountered: during vagus stimulation and even when recovery had brought diastolic pressures back to the normal range, all velocities were too low to fall on any continuation of the curves obtained before manipulation of the nerve. Of the controls before each stimulation, some were up in the previous normal area, while others (with electrodes already on the nerve and possibly without sufficient recovery from previous stimulation) were down with the low velocities.

The number of points was great enough to give statistical significance to the magnitude of their displacement, and suspicion that the animal had deteriorated was allayed by the consistency of velocities in the thoracic segment. This vagus portion of the two curves has therefore been included in the figure in the form of broken lines, as indicated. The experiment as described has not been repeated on additional dogs, but the diagrams shown here give the results of three separate stimulations of the vagus with rest between them.

Analytical review of some earlier experiments revealed independent and quite different confirmatory evidence of this apparent specific effect of vagus stimulation upon pulse wave velocity in addition to its depressor action. Here it was demonstrated at higher pressures in the thoracic aorta. In a previous paper (6) there was described a procedure by which the aorta was occluded internally, causing a considerable increase in diastolic and mean pressures above the occlusion. The entrance of the vagus phase of the reflex depressor response to the rise in pressure was identified by the lengthening of the pulse cycle. When the velocities of successive pulses were determined and plotted against the diastolic pressures, the events were shown to be as pictured in figure 3, where the time sequence of cycles is indicated by the straight lines joining points.

The diastolic pressure and pulse velocity rose with occlusion; but even as the pressure continued to rise the pulse wave velocity fell off when depressor activity set in. The velocity remained lower than that expected until removal of the occlusion released the vagus tone. Analysis of two such occlusions gave practically identical results.

Nonidez (8) has published some of his studies on the systematic mapping of the innervation of the aorta; but so far as we can find there is at present nothing definite in the literature to suggest a histologic basis for these last results. Such differences in pulse wave velocity can be brought about only through variation in the elastic properties of the aortic wall. The most obvious hypothesis would involve a change in the tone of the smooth musculature, evoked by either nervous or hormonal influences. Identification of the specific factors and their reconciliation with anatomical findings will have to rest with extension of our present researches and those of Nonidez.

SUMMARY

1. Derived from records obtained by previously described methods, continuous curves are presented which show the changes in pulse wave velocity from aortic arch to femoral artery in seven dogs.

2. The wave is shown to accelerate quite evenly over this range, with considerable variation in the rate of acceleration in different dogs.

3. Measurements of the elasticity of rings cut from an aorta give results which are consistent with such an acceleration.

4. The pulse wave velocity corresponds to different functions of the diastolic pressure in the thoracic and abdominal portions of the aorta.

5. Stimulation of the vagus nerves, whether electrical or reflex, is accompanied by a slowing of the pulse wave in addition to that produced by the lowering of the diastolic pressure. In the records available so far, this effect is evident at low and normal pressures in the abdominal aorta and at higher pressures in the thoracic aorta. The only hypothesis that can be put forward in explanation at present is that with vagus stimulation either nervous or hormonal influences bring about a change in the elasticity of the arterial wall by varying the tone of smooth muscle fibers.

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KIDNEY FUNCTION IN ADRENAL CORTICAL INSUFFICIENCY[1]

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One of the most constant findings observed in animals suffering from adrenal cortical insufficiency is the abnormal concentration of several of the blood and urinary constituents (9). According to some investigators, these abnormalities are referable to a primary renal dysfunction which is attributable to the absence of the vital cortical hormone; others relate them to the loss of normal cell permeability to certain ions or to disturbances in the circulation (23). Since the electrolyte and water disturbances are such fundamental manifestations of adrenal cortical insufficiency, it is important to determine the exact nature of the *renal-adrenal* relationship.

In this paper, observations are recorded on the morphology of the kidney (and liver) of adrenalectomized rats, cats and dogs under different conditions. Attempts were made to investigate histochemically in rats suffering from different degrees of insufficiency: 1, the passage of water through the glomerulus, and 2, the reabsorption of water in the descending limb of the loop of Henle. In addition, an investigation was made of the alleged functional relationship between the adrenal cortex and the posterior pituitary.

Histological appearance of kidneys and liver of adrenalectomized rats, cats and dogs. The kidneys of three series of animals were studied: 1, fourteen one-month-old rats manifesting various degrees of adrenal cortical insufficiency 5 to 7 days after complete adrenalectomy; 2, ten adult rats in various stages of adrenal insufficiency, following bilateral adrenalectomy; and 3, three dogs and three cats that were maintained in good clinical condition with cortical extract[2] for 100 days following bilateral adrenalectomy, and were then allowed to die of adrenal insufficiency on withdrawal of treatment. Equatorial slices of the kidney were fixed in formalin-Zenker's fluid and sectioned at 5 μ. The sections were stained with hematoxylin and eosin or with Mallory's triple stain.

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

[2] The adrenal glands used in the preparation of these extracts were generously supplied by Sharpe and Dohme, Philadelphia, and by Mr. Robert L. Fox, of Baltimore. The extracts were prepared by the methods described elsewhere (9).

Microscopic examination of the tissues from the above-described three groups of animals showed a complete absence of any significant pathological changes in the kidney or the liver. One area of fibrosis in the medulla of the kidney and several minute areas of lymphocytic infiltration in the liver of rats were obviously accidental changes which could be observed also in preparations from normal control animals.

Our findings that the kidneys are not altered anatomically in experimental adrenal insufficiency is contrary to the claims of several recent investigators. Simpson and Korenchevsky (21) conclude that after adrenalectomy there is an extensive microscopically visible injury in the rat's kidney, presenting evidence in a low-power photomicrograph of the renal "cortex" showing pale-staining areas interspersed among the normal dark-staining cortical tubules. Careful examination of their figures will show that the pale-staining areas in the cortex of the experimental rats are not areas of injury. They are, rather, areas of normal, lighter staining ducts and loops of Henle such as might easily be incorporated in a tangential section of the cortex close to or slightly encroaching on the medulla.

Banting and Gairns (2) reported general hyperemia, focal areas of hemorrhage, changes in the cytoplasm, and albuminous and cellular casts present in the kidneys of dogs shortly after adrenalectomy. These severe changes must be attributed to factors, other than adrenal insufficiency, that are vaguely included in the term operative shock. Our studies were made on dogs which were allowed to die of cortical insufficiency after they had been maintained in a healthy state for 100 days following complete adrenalectomy by the administration of cortical extract. This long post-operative period was an effective protection of the animals against operative shock.

Marshall and Davis (18) and Swingle (23) find no noteworthy pathology while Hartman and his collaborators (12), Marine (17) and McMahon and Zwemer (15), stress the occurrence of an "acute tubular nephritis" and "lipoid nephrosis" in adrenalectomized cats. The latter regard as particularly significant the presence of large numbers of lipoid droplets in the cells of the proximal convolution, the loop of Henle, and in the lumina of the renal tubules. However, this extensive distribution of lipoid in the kidney has been shown to be perfectly normal in cats in a certain stage of their life cycle; *e.g.*, during pregnancy (20). The "acute tubular nephritis" was absent in the cats which we studied. As in the case of the profound changes reported in dogs, this "nephritis" must be regarded as evidence of operative shock, particularly in view of the short period of survival of these animals in the hands of these investigators.

The absence of any clear morphological evidence of significant liver damage in our animals is contrary to the results reported by Banting and

Gairns (2). The degeneration of the liver cells observed by these workers must be attributed to operative shock, which was excluded in our experiments. However, the normal microscopic appearance of the liver does not contradict the fact that there may be marked hepatic dysfunction. That this does in fact occur is demonstrated by studies on the carbohydrate metabolism (9, 10).

Histochemical studies on the passage of water through the renal nephron in adrenalectomized rats. The method used for these studies (4, 5, 7) involves the intravenous injection of a solution of sodium ferrocyanide. After a variable time, the rats are killed and the kidneys are removed. Equatorial slices of the kidney are frozen in liquid air and dried in a vacuum at −30°C. Sections of these tissues are tested histochemically for ferrocyanide. The location of the Prussian blue which is visible with the microscope shows accurately the relative amounts of ferrocyanide present in different portions of the renal nephron. Since ferrocyanide is eliminated through the glomerulus without any evidence that it is secreted or reabsorbed by the cells of the renal tubule, it becomes possible, by comparing the sites where ferrocyanide is more concentrated, to determine precisely the portions of the renal nephron where water is being reabsorbed, and to what degree this occurs. Two variations of this method were employed. In the first, the rats were killed 10 minutes after being injected with a small amount of ferrocyanide. This was designed to show more specifically the nature and extent of the reabsorption of water in the loop of Henle and elsewhere, using the concentration of ferrocyanide as a measure of this function. In the second procedure, the rats were killed 30 seconds to 4 minutes after the injection of a large dose of ferrocyanide. This was designed to show specifically the number of glomeruli functional at the moment in the kidneys of rats suffering from different degrees of adrenal insufficiency.

The effects of anesthesia and operative technique were excluded by the use of the following controls: 1, normal unoperated litter mates; 2, rats in which a fragment of the adrenal had been left at the time of operation; 3, rats maintained in clinically good condition by the administration of cortical extract; and 4, rats treated for 10 days with cortical hormone and then permitted to go into adrenal insufficiency by stopping the treatment.

Reference to table 1 will show that during the first 24 hours after adrenalectomy there is no clear change from the normal in the reabsorptive function of either the terminal portion of the proximal convolution or the descending limb of the loop of Henle. The only change appears later in early or moderate insufficiency in young rats (table 1) or in marked insufficiency in adult rats (table 3). At these times there is an abnormal reabsorption of water in the loop of Henle. Table 2 shows that still later, when the animal is prostrate, there is a cessation of the passage of water

TABLE 1

The distribution of ferrocyanide in the lumen of the nephron of month-old rats after the injection of a small dose of this salt

All rats were injected in the tail vein with 0.25 cc. of a 10 per cent solution of sodium ferrocyanide. Ten minutes later, no ferrocyanide was present in the glomerular space in amounts large enough to be detected. The amount of ferrocyanide visible in the different portions of the renal nephron, hence the degree of water-reabsorption in these sites, is indicated in the last two columns.

NO. OF RATS	CLINICAL CONDITION	CONCENTRATION IN TERMINAL PORTION OF PROXIMAL CONVOLUTION	CONCENTRATION IN DESCENDING LIMB OF LOOP OF HENLE
4	Unoperated control	0, 0, 0, tr	±, +, +, ++
1	Incomplete adrenalectomy	tr	±
3	Complete adrenalectomy, treated. Clinically normal or very early insufficiency	0, 0, tr	++, ++, +(+)
1	Complete adrenalectomy. No symptoms of insufficiency	±	+++(+)
2	2 hours post-adrenalectomy	0	+, +(+)
2	5 hours post-adrenalectomy	0	+, +++
2	12 hours post-adrenalectomy	0	+, ++
2	24 hours post-adrenalectomy	0, tr	+++, +++
3	Early or moderate insufficiency	0	tr
3	Early or moderate insufficiency	0	±
2	Early or moderate insufficiency	0, tr	+
5	Early or moderate insufficiency	0	++
4	Early or moderate insufficiency	0, 0, tr, tr	++(+), +++, +++, +++(+)
1	Treated for 10 days. Early insufficiency three days after withdrawal of treatment	tr	±
1	Prostrate	0	0

through the glomeruli. These disturbances in the elimination of water by the kidneys of rats suffering from adrenal cortical insufficiency are definitely demonstrable in spite of the absence of significant morphological indications of nephron injury. This knowledge of demonstrable functional

change in the kidney, when interpreted in the light of known changes taking place in the composition of the urine and other body fluids under the same conditions, permits us to draw certain conclusions concerning the rôle of the kidney in adrenal cortical insufficiency.

In early stages of adrenal cortical insufficiency, there is a decrease in the sodium and chloride in the blood, which is compensated for by a corresponding increase in potassium, urea and bicarbonate. The result is that the total molar concentration of the plasma of such animals does not deviate from that of normal animals (9, 13).[3] At the same time, sodium and chloride appear in the urine in greater amounts than normal, while potassium, urea and bicarbonate appear in smaller amounts than normal (11). These very fundamental changes in the composition of the blood and urine must be attributed either to dysfunction of the renal nephron which is no longer able to reabsorb sodium and chloride as it does normally and prevent the reabsorption of potassium, urea and bicarbonate or to some at present unknown, and in the authors' opinion highly improbable, extra-renal factor.[4]

The dysfunction of the renal nephron assumed above occurs in the absence of any demonstrable histological lesion of the kidney. This dysfunction in the renal nephron will initiate a complex chain of reactions involving a balance between 1, the amount of water and salts ingested by the animal; 2, the amount lost through the skin, bowels and lungs; 3, the shift of water and electrolytes between blood and extracellular spaces; 4, the shift between the extra- and intra-cellular spaces. The picture is thus so complex that any dogmatic description of the details of the sequence of events must be open to very serious question. It may be assumed, however, that the abnormally great reabsorption of water which we have observed in adrenal insufficiency represents a physiological compensatory mechanism whereby a fatal loss of body fluid is prevented.

In the last stages of adrenal cortical insufficiency new factors appear that affect the kidney in a very definite way. There are, for example, changes in carbohydrate metabolism, in oxygen consumption, in various endocrine glands, and in blood volume. The last-named extrarenal factor operates terminally in reducing the blood pressure. In this severe condition there is a great decrease in the amount of glomerular fluid formed per unit of time, and in the number of glomeruli which are active. This culminates finally in the complete cessation of the passage of water across the glomerular membrane (see table 2).

Gömöri and Podhradszky (8) have recently promoted the view that the

[3] The contrary results of Margitay-Becht and Binder (16) include data for normals which deviate far from generally accepted values (19).

[4] For a more detailed description of the sequence of events which correlate the observed changes in the body fluids with renal changes see reference (9), chapter XI.

azotemia observed in adrenal insufficiency is due to hemodynamic factors which cause a diminution in the volume of the glomerular fluid. Azotemia, however, is present at an early stage of insufficiency before circulatory disturbances are prominent (9, 18), while, as shown in the present work, the glomerular changes do not occur until the animal is almost prostrate.

The results presented in this paper can be explained best by the view that in adrenal insufficiency the kidney is responsible for the loss from the

TABLE 2

The distribution of ferrocyanide in the lumen of the nephron of month-old rats after the injection of a large dose of this salt

All rats (with the exception of the last in the table) were injected in the tail vein with 0.3 cc. of a 20 per cent solution of sodium ferrocyanide. The exceptional rat was injected intravenously with 1 cc. of 0.85 per cent sodium chloride solution followed ten minutes later by the intravenous injection of the usual amount of sodium ferrocyanide.

NO. OF RATS	CLINICAL CONDITION	TIME AFTER INJECTION	GLOMERULUS	CONCENTRATION IN	
				Proximal tubule	Decending limb of loop of Henle
		min.			
1	Unoperated control	½	0	0	0
1	Unoperated control	1	+	+	+
1	24 hours post-adrenalectomy	½	0	0	0
1	24 hours post-adrenalectomy	1	0	*	±
3	Marked insufficiency	1	0	0	0
1	Marked insufficiency	1	About ¼ = 0 About ¼ = tr About ½ = +	*	0, absent
1	Prostrate	1	+ in most glomeruli	tr	++
2	Prostrate	2, 4	0	0	0
2	Prostrate	1	+	*	0, absent
1	Prostrate	1	0	0	0

* Present only in proximal portion; absent from distal portion.

organism of sodium, chloride and water. The view expressed by certain authors that the observed disturbances in the water and salt balance of the body are due solely to shift of water from the extracellular to intracellular spaces or from the plasma through the capillaries, is contradicted by a number of well-established facts (9, 13). The shifts which occur between intracellular, extracellular and plasma fluids are more logically to be attributed to secondary effects induced primarily by renal dysfunction.

The relation of the adrenal cortex to the neurohypophysis. Our observation

of an abnormally great rate of reabsorption of water in the tubule in late stages of adrenal insufficiency suggests the possibility of some inverse relation between the activity of the adrenal and the neurohypophysis. Posterior pituitary liquid has been shown (3, 5) to exert its antidiuretic effects by stimulating the reabsorption of water in the thin portion of the loop of Henle (and the terminal portion of the proximal convoluted tubule). Superficially, therefore, the picture in later stages of adrenal insufficiency resembles that observed after injection of posterior pituitary liquid. Moreover, one might argue that the posterior lobe of the hypophysis is

TABLE 3

The distribution of ferrocyanide in the lumen of the nephron of adult rats some time after the injection of a small dose of this salt

All rats were injected in the tail vein with 0.5 cc. of a 10 per cent solution of sodium ferrocyanide. Ten minutes later, no ferrocyanide was present in the glomerular space in amounts large enough to be detected. The degree of reabsorption of water in the terminal part of the proximal convolution and in the descending limb of the loop of Henle are indicated by the amounts of ferrocyanide visible in these sites.

NO. OF RATS	CLINICAL CONDITION	CONCENTRATION IN TERMINAL PORTION OF PROXIMAL CONVOLUTION	CONCENTRATION IN DESCENDING LIMB OF LOOP OF HENLE
2	Unoperated control	0	+
1	Partial adrenalectomy	0	+
2	Complete adrenalectomy. Treated. Clinically good	0	+
3	Early insufficiency	0	±, ±, +
3	Early insufficiency	Most nephrons, 0; some nephrons, tr	±, ±, +
1	Marked insufficiency	0	±
1	Marked insufficiency	0	+(+)
3	Marked insufficiency	tr	+++

stimulated to activity in adrenal cortical insufficiency as a mechanism whereby water would be conserved for the organism (22).

In view of the above considerations the neurohypophyses of a series of rats dying of adrenal insufficiency have been examined by the method recently described by one of us (6). By this method it is possible to observe the parenchymatous glandular cells which appear to secrete the antidiuretic substance and to determine their probable state of activity. In this material there was found no evidence, however, of any stimulation of the glandular cells as a result of adrenal cortical insufficiency. Unlike other conditions in which there is need for conservation of body water (thirst, exposure to heat, *etc.*), the neurohypophysis is not stimulated in adrenal insufficiency.

Winter and his co-workers (24) have recently suggested the possibility of an interrelationship between the adrenal cortex and the posterior lobe of the pituitary. They base their conclusion on the fact that in spite of the usual increase in the concentration of potassium there is no marked decrease in the concentration of sodium or chloride following adrenalectomy in cats with diabetes insipidus. In view of our own experiments it would be more logical to consider the changes observed by Winter *et alii* as the resultant effects of a complex series of changes which incidentally result in relatively slight changes in the sodium and chloride content of the blood. It is unnecessary to assume that the adrenal cortex and the neurohypophysis are involved in any direct interrelationship.

SUMMARY

1. Histological examination failed to reveal any significant structural changes in the kidney (and liver) of rats, cats and dogs, which could be attributed to the effects of adrenal cortical insufficiency. The contrary results of previous workers are shown to be based on misinterpretations of their data or to be due to the effects of operative shock.

2. The results of histochemical studies of the kidney of rats in early stages of adrenal cortical insufficiency demonstrate no clearly visible morphological or functional pathology. During marked insufficiency there is a demonstrable increase in the rate of reabsorption of water through the tubule. In the late stage of insufficiency the passage of water through the glomerular membrane is slowed down or ceases entirely.

3. The rôle of the kidney in adrenal cortical insufficiency is discussed on the basis of the above mentioned findings and the known changes occurring in the composition of the blood and urine.

4. Study of the neurohypophysis of rats dead of adrenal insufficiency indicates the absence of any close *adrenal-neurohypophyseal* interrelationship.

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MEASUREMENTS OF WATER DRINKING IN DOGS

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Certain quantitative features of drinking are described in dogs subjected to diverse amounts of water abstinence. Two kinds of drinking were studied; one in which the water did not reach the stomach because the esophagus had been rendered fistulous by surgical operation, and the other in which the water reached the stomach and so furnished water to the body.

By means of the fistula of the esophagus, the drinking and the disappearance of the urge to drink were made independent of one another. In separating these two it was then possible to measure drinking both in the steady state of water depletion as well as at the sudden conclusion of abstinence. The results indicate that precise correlations exist between the body's water deficit and the water drinking under standardized conditions.

METHODS. Fistula of the esophagus was produced in two dogs (by Dr. R. T. Bellows) using the surgical procedure described by Bellows and Van Wagenen (1938); under ether anesthesia the esophagus was brought outside the skin surface on the ventral portion of the neck. After two days the esophagus ruptured so that a discontinuity was produced between mouth and stomach. The two dogs were kept in health for 5 and 11 months.

All food and water were furnished by inserting a funnel at each meal into the lower portion of the esophagus. Water taken by the dog from a drinking pan, and whatever saliva was formed, fell from the upper end of the esophagus. There was no drainage from the upper into the lower esophagus. No saliva reached the stomach or was furnished to the alimentary tract during these months. A comparable condition for water drinking was described by Bernard (1856) who produced esophageal fistula in a horse and gastric fistula in a dog, and observed that drinking continued intermittently (when desiccated) so long as the fluid escaped through the fistula.

The fistulous dogs were kept in metabolism cages so modified that water was drunk in a side compartment to which only the head and neck were admitted. As water was taken from the drinking pan it fell from the

neck into another container where it was measured. At the end of each draft of water the dog withdrew its head, when residual quantities of fluid escaped from the esophagus and collected with the urine.

Continuous records of water drinking were obtained by registering on a kymograph the excursions of a float placed in the receptacle for the fistula water. For the normal dog, water drinking was recorded from a float in a pan connected on a level with the dog's drinking pan. Other records were made in the presence of an observer, by noting the periods in seconds during which the dog drank and the number of gulps with which drinking occurred.

The diets used had to be liquid when introduced into the esophagus of the fistulous dogs, and such that relatively small quantities of water secured this liquid condition. Equal parts of "fox chow" and whole dried milk were suspended so that as much as 2.5 Calories could be given with each cubic centimeter of water. Four normal dogs received similar diets, but usually mixed with still smaller quantities of water. Feedings were given twice a day, about 9 a.m. and 4 p.m. When large quantities of water were to be administered to fistulous dogs, additional waterings were interpolated.

The dogs were weighed just preceding each morning feeding, on a balance sensitive to 20 grams. Upon the accuracy of the weights depended the estimates of water privation. No corrections were made for retentions from day to day in order that the estimates of *A*, water balances, and *B*, water exchanges would be entirely independent of one another. Urine volumes and specific gravities were obtained each 24 hours, but the dogs were not catheterized; exact periods were not necessary since only averages of urine volumes were employed.

Manner of drinking. Water drinking was studied in two conditions: *a*, in the steady state; which in turn could be regarded as of two sorts, *a1*, sham-drinking in the fistulous dog, and *a2*, *ad libitum* ingestion in the normal dog; *b*, sudden drinking following denial; also of two sorts, *b1*, sham-drinking, and *b2*, real ingestion.

The most constant feature of drinking was the number of gulps per minute made by the animal. The frequency of gulping decreased slightly as water was ingested; this could possibly be called a fatigue in the neuromuscular apparatus involved.

Various features of drinking are presented in table 1; only maximal drafts can easily be compared. A draft of 6900 cc. in dog B means that a quantity equal to almost half the body weight was passed through the mouth without interruption. A draft of 1800 cc. or one-tenth the body weight in the non-fistulous dog D means that the alimentary tract accommodated this much, since there was no time for appreciable absorption within the 3 minutes spent in ingestion.

The velocity of continuous drinking might be only one-third of the maximum, either toward the end of a prolonged draft, or when only a small water shortage prevailed. The volume taken in each gulp might be only one-fourth of the maximum.

a1. The frequency of drinking in fistulous dogs varied with times of day. With moderate desiccation, drinking at night was rare; but in severe privation almost as much drinking occurred at night as in daylight hours.

The same mean rates of discontinuous drinking and the same mean frequencies of drafts prevailed throughout five months. No regular changes occurred in successive days at the same water deficit. The only definite conditioning that was noted was the tendency to ingest water oftener in the presence of an attendant. A fistulous dog with a slight water privation might drink at no other time throughout the day. Since,

TABLE 1

Maximum quantities of water drunk

	DOG A	DOG B	DOG B	DOG B	DOG C	DOG C	DOG D	DOG E
	Condition							
	Normal	Fistulous (spring)	Fistulous (autumn)	With connector	Normal	Fistulous	Normal	Normal
Body weight, kgm.	18.4	13.4	14.9	15.0	15.8	15.0	17.4	22.7
Cc. per draft	950	2100	6900	1620	950	400	1800	1840
Minute per draft	2	4	10	3	3	3	3	3
Drafts per hour	3	5				30		
Drafts per day	15	39						
Cc. per minute	400	470	410	510		250	730	700
Cc. per gulp			2.5	2.5		2.0	4.1	4.8
Gulps per minute			164	170		160	190	170

however, the dogs were subjected to the same stimuli when in water balance, such factors were controlled.

When severely desiccated, the fistulous dogs spent 20 per cent of the time over periods of one hour in drinking. Over periods of 24 hours, up to 10 per cent of the time was so spent; hence a very appreciable amount of the dog's daily effort went into drinking. In the most extreme instance 71 liters of water (4.7 times the body weight) passed through the mouth in 24 hours.

When desiccated by 4 per cent of the body weight, dog B sham-drank 300 to 1000 cc. at each draft (mean 710 cc.); dog C only 40 to 100 cc. The intervals between drafts and the volume passed per minute of continuous drinking (fig. 1) were the only significant differences found between the two fistulous dogs. Rates of sham-drinking represent frequency × vol-

ume, which is here a constant product analogous to rates of pulmonary ventilation.

a2. When water was continuously available, normal dog A on nearly dry diet drank about 50 cc. (0.3 per cent of body weight) at each draft, and took drafts approximately every two hours of daylight. More than average ingestion occurred within three hours after food, as Kleitman (1927) and Gregersen (1932) also reported. Cows (Atkeson and Warren, 1934) regularly drank 7 to 10 liters at a draft (0.9 to 1.5 per cent of body weight).

b1. Fistulous dog B was subjected to various brief periods in which drinking-water was not available, to see whether water not obtained for sham-drinking would be sham-drunk when water was again available.

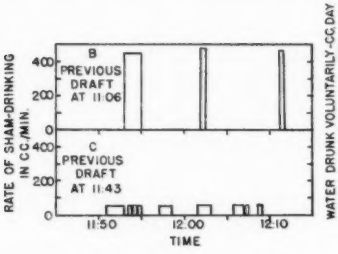

Fig. 1

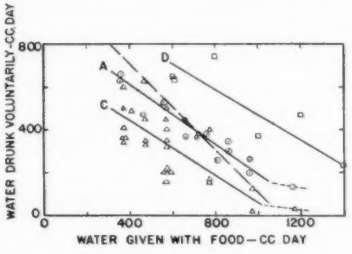

Fig. 2

Fig. 1. Rates and frequencies of sham-drinking in two dogs with esophageal fistula. Dog B had a water deficit of 3.7 per cent of the body weight and drank 1750 cc. per hour. Dog C had a deficit of 4.5 per cent and drank 1940 cc. per hour.

Fig. 2. Amounts of water voluntarily drunk per 24 hours in each of three dogs, when various quantities of actual + potential water were given with the food. The dashed line indicates the slope of the correlations if the total water ingested had been independent of the amount given with food.

Within limits this proved to be the case; but after more than one hour of privation, sham-drinking no longer made up for that missed (see also Bellows, 1939).

b2. Within four hours of water privation, normal dogs not recently fed drank less than 0.1 per cent of the body weight per hour, and usually refused water offered at hourly intervals.

Daily balances and requirements. The amounts of food actually received (table 2) were constant within about 2 per cent each day. This constancy was secured because the normal dogs, except on 2 or 3 days, ate all of the food offered, and the fistulous dogs inevitably received the food into the stomach. Occasionally slight losses from the esophageal fistula occurred through subsequent stomach movements, the food appearing to splash from the intensely moving stomach.

The amounts of water taken in and put out in the average day during which the dogs were each in water balance, are indicated in table 2. Water balance was said to prevail when the fistulous dog drank less than one liter of water by mouth overnight; in the normal dog it prevailed on any day when water was being taken *ad libitum*.

TABLE 2

Metabolic data for 24-hour periods

	DOG A	DOG B	DOG B	DOG B	DOG C	DOG C	DOG D
	Condition						
	Normal	Normal	Fistulous (spring)	Fistulous (autumn)	Normal	Fistulous	Normal
Mean weight, kgm.	18.4	15.2	13.4	14.9	15.8	15.0	17.4
Days studied	62	8	66	58	63	35	28
Diet:							
"Klim," grams	140	117	150	150	150	150	0
"Chow," grams	140	117	150	150	150	150	400
Calories	1175	980	1260	1260	1260	1260	1320
Cl. m.-eq.	52	43	55	55	55	55	60
Water, cc.	20	16	21	21	21	21	45
By oxidation, cc.	140	117	150	150	150	150	155
Mean drunk, cc.	920	877	1280	1114	750	1170	1060
Mean total water taken, cc.	1080*	1010*	1450	1285	920*	1340	1260*
Mean urine, cc.	430		252	262	168	239	390
Mean urine sp.gr.	1.032		1.034	1.046	1.050	1.032	1.030
Surface area, sq.m. (computed)				0.68	0.72		
Water required in desiccation, cc.	680		1420	1190	735	1200	
Evaporation in desiccation, cc.	530				605		
Mean "evaporated," cc.†	650		1200	1025	685	1100	870
Mean "evaporated," per cent of heat	32		55	46	31	50	38
Mean urine in desiccation, cc.	150				130		205
Maximal urine sp.gr.	1.081		1.044	1.064	1.087	1.044	1.058
Mean water in feces, cc.				150		115	

* When 600 cc. of it was contained in food as eaten.

† Total intake minus urine. Includes feces, saliva, etc.

Several inconstant factors could not be avoided, even though the program would appear to have furnished uniform conditions. Amounts of fluid lost in feces and in evaporation from the mouth were unavoidably variable. In the fistulous dog additional factors were rare regurgitation, loss of saliva from the upper esophagus, and the wetting of the neck region

by sham-drinking or by licking with the tongue. Temperature conditions were uniform throughout most of the periods of observation, so that the amounts of water required for cooling were uniform, at least on successive days within which water balance was repeatedly restored and upset.

The mean water requirements (table 2) were approximately 1 cc. per Calorie of food, varying from 0.73 to 1.15. Quite similar ratios prevail in man and other large mammals that have been studied.

The variability of the body water content may be judged by the daily differences of body weights (0.7 per cent upon successive days) when the normal dogs were kept in water balance. Variability of turnover may be indicated by the daily differences in amounts of water simultaneously ingested *ad libitum* (8 per cent) and excreted (in urine 35 per cent, and in evaporation 20 per cent). The variabilities of turnover hence prove to be small enough so that several factors can be shown to modify the requirement and output significantly.

The individuals that were used (table 2) showed significant differences in water requirement and in urine output between normal and fistulous periods. Upon esophagostomy, the water requirement increased by about 50 per cent, remaining there for a month or more and gradually declining in both dogs. This surgical procedure was not the only factor influencing the water requirement, for dog B was found to increase its requirement in two later cycles. Part of the difference after esophagostomy might result from the loss of saliva and from evaporation from the esophageal surfaces. Another part may be due to the new criterion used in judging whether water was required. In addition, the esophageal fistulous dog was unable to take water *ad libitum*, hence its water was furnished at definite periods and was not available during the 24 hours as it was in the normal dog.

This point was further amplified by certain studies in the normal dog (fig. 2). It is seen that it was difficult to furnish enough water simultaneously with the food so that *no* water was drunk at any other time. The water requirement therefore depended upon whether water was given at some fixed time or whether the water was available at all times; and diverse water requirements were comparable (table 2) only when a fixed amount of water was given with food. Spiegler (1901) demonstrated that a young dog drank less and grew less when denied water for a few hours following each meal.

When the dogs were deprived of water, less water was required for maintenance, for when water was re-administered, a smaller amount was necessary to restore balance than would have been drunk if water had been constantly available throughout the same number of days (fig. 3). This may represent, in part, the above factor of *time* at which water be-

comes available. There was a sparing of water, particularly in urine and feces, and more in dog A (400 cc. per day) than in others. Such a smaller water requirement in desiccation was observed by Straub (1899). To some extent a similar economy prevailed in the fistulous dogs. In the recovery from desiccation it has been found by Straub, and others, that extra water is excreted and that there is an enlarged excretion of chloride, nitrogen, and other solutes.

Hence the conclusion may be drawn that there is no single water requirement in the dog. It depends upon the procedure chosen and the

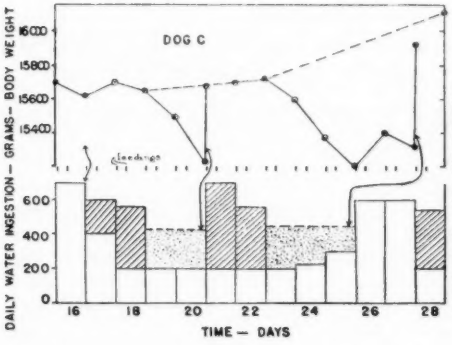

Fig. 3

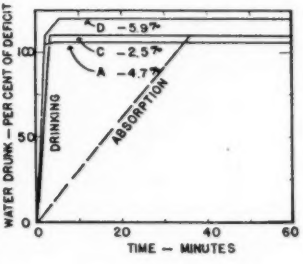

Fig. 4

Fig. 3. Protocol of two water-privation tests. Body weights indicate water deficits. Ingestions were of three kinds; open rectangles, water mixed with food; hatched rectangles, water drunk when allowed *ad libitum* throughout the 24 hours; stippled rectangles, water drunk within 10 minutes at the end of a privation. The water that was suddenly ingested at the close of day 27 is referred to days 23 to 25 when privation was most severe.

Fig. 4. Cumulative water ingestions in a selected test upon each of three normal dogs. On the days represented, the water deficits were 5.9, 2.5, and 4.7 per cent of body weights. Water absorption from the alimentary tract is inferred from the data of Klisiecki et al. on dogs that had not been deprived of water, given 2.5 per cent of their weights of water.

conditions. In comparing results even on the same individual dog it is therefore continually necessary to inquire whether the procedures and criteria were the very same in obtaining the measurements to be compared.

Sham-drinking. To relate the rates of discontinuous sham-drinking on various days with the amounts of water of which the animal had been deprived, it was necessary to choose a suitable measure of water deficit. Three bases were available: *1*, the loss of body weight, which might be taken to represent shortage of water so long as food intake and other factors were constant; *2*, the amount of water required to maintain the

animal in balance minus the amount of water actually given; *3*, the amount of water subsequently used to make up the deficit (restore the water balance). Basis *1* ("weight deficit") was the only method by which deficit could be judged independently of water exchanges, and hence was used for the chief comparisons to be drawn. Basis *2* ("requirement deficit") gave deficits too large, since the water requirement during desiccation was less than during water balance. Basis *3* ("dietary deficit") was applicable in the case of the fistulous dogs since these dogs were given water by

TABLE 3

Water balances of fistulous dogs

The metabolic periods ended at 9 a.m. each day. Urine was contaminated by sham-drunk water. "Water given" includes 150 cc. formed by oxidation.

DOG	24-HOUR PERIOD 1937	FOOD GIVEN	BODY WEIGHT	WEIGHT DEFICIT	WATER GIVEN	WATER DEFICIT OF DIET	SHAM-DRUNK	URINE
		grams	*grams*	*grams*	*cc.*	*cc.*	*cc.*	*cc.*
B	Oct. 7	300	14,900	0		0	880	
	8	300	14,630	190	1,270	40	8,950	265
	9	300	14,340	430	870	480	36,200	270
	10	300	13,910	810	1,170	620	17,500	(630)
	11	300	14,450	240	1,770	160	10,500	(560)
	12	300	14,580	40	1,270	200	32,300	(410)
	13	300	13,840	760	870	640	5,170	(480)
	14	300	14,530	0	2,120	−170	0	500
	15	300	14,480	0	1,310	0	0	165
C	Dec. 9	300	14,910	0		0	300	520
	10	300	14,800	80	1,270	40	3,470	(600)
	11	300	14,540	300	1,270	80	2,550	(420)
	12	300	14,430	390	870	520		170
	13	300	14,140	590	870	960	25,200	
	14	300	14,570	170	1,920	350	2,500	(570)
	15	300	14,710	0	1,660	0	790	240

tube until the urge to drink disappeared, hence the water taken to relieve the deficit did not represent choice on the part of the animals.

Table 3 shows examples of water turnover in 24-hour periods. Constancy of body weight was succeeded by decrease of weight, followed by regain when enough water was again furnished. The basal body weights were corrected on successive days for the trends which were apparent between initial control days and final control days.

Several successive deficits were represented during the program of a single water privation. While great variabilities occurred in the rates of discontinuous sham-drinking upon days of supposedly equal deficit in body weight, it is allowable to average the rates upon such days. It is

seen that the mean amounts drunk (fig. 5) are proportional to the weight deficits. In the 84 days of water deficit, the deficit exceeded 2 per cent of the body weight upon 46 of them; on these days the amounts drunk were such that the deficit would have been made up every 40 minutes throughout the 24 hours.

A similar relationship is found in the correlation of the same amounts of water ingestion with the deficits of water as estimated from the diets (basis *3*). While the mean "dietary deficits" are estimated to be larger than the "weight deficits" (basis *1*, fig. 5), some individual deficits are smaller.

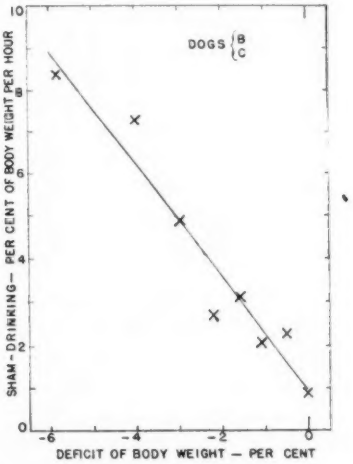

Fig. 5. Correlation of mean rates of sham-drinking with water deficits (as estimated from body weights). Measurements of drinking were averaged from 24-hour periods. Among 84 days of water deficit, the rates were averaged in groups of 12 points taken in the order of the amount of deficit.

There was no significant difference in the average rates of discontinuous water drinking in 24-hour periods between these two dogs, nor in dog B as studied in two ranges of time separated by five months.

Real ingestions. The largest weight deficit observed on the constant diet was 7.4 per cent, and the longest privation lasted 4 days. Spiegler (1901) observed a loss of 9.7 per cent of the weight in a dog fed without additional water on meat and fat for 12 days; Straub (1899) produced 9.3 per cent loss in 3 days; and Mayer (1901) reported still larger losses.

When a normal dog was offered water at the end of a privation period the drinking was immediate, rapid, and ended abruptly (fig. 4). Nearly the entire amount of water was taken within two or three minutes, de-

pending upon the time necessary to swallow it at the maximal rate. Among the four dogs tested there were no significant differences. All were precise in taking water, up to a time when they suddenly became indifferent to it. Water reoffered in the sixth to tenth minutes of recovery added only 7 per cent to the ingestion, and only 3 per cent further was taken within 60 minutes.

The amounts ingested are correlated with mean deficits of body weight (fig. 6), slightly exceeding them by an average of 17 per cent where the deficits exceeded 2 per cent. This slight excess might be within the limits of error in determining the deficits. It might also correspond to amounts

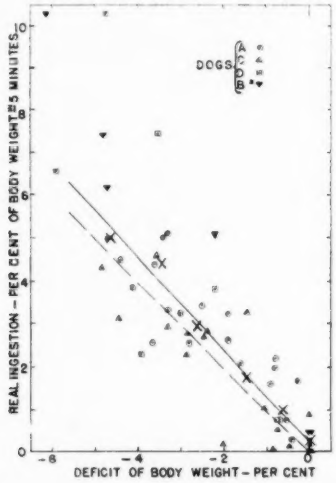

Fig. 6. Correlation of amounts of water drunk with water deficits (as estimated from body weights). Among 41 tests plotted, the amounts drunk were averaged (×) in groups of 8 points taken in the order of the amount of deficit. Dog B* was fistulous and in these six tests drank with the connecting tube in place; these points are not included in the averages.

of solute retained in the body which now required excess water for their excretion (Straub, 1899). The excesses of water drunk were on the average only 0.4 per cent of the body weight.

A few additional measurements of ingestion, at the end of desiccation, were made in dog B into whose esophageal segments a connecting tube was inserted for the occasions. The four (or six) points so obtained have been added to figure 6, and show a hardly significant tendency to exceed the ingestions of the other three dogs.

Within the time occupied by ingestion, practically no absorption of water from the alimentary tract was possible. Klisiecki et al. (1933)

showed that absorption of an amount of water equal to 2.5 per cent of dogs' weight was nearly proportional to time during 35 minutes. Absorption might take longer times when larger volumes were drunk, or shorter times in desiccation. Best and Cohnheim (1910) noted on a dog with both esophageal and gastric fistulas that after water abstinence, water left the stomach sooner than usual. In two instances water ingestion appeared to cease before the unusually large deficits were fully made up, for on being offered water at a later time of day (as was often done) considerable further quantities were taken. In one of these instances, dog B with the connecting tube in place continued for a minute to drink water although the stomach became so full that the water leaked out around the lower end of the connecting tube as fast as it was swallowed. Apparently the fulness of the stomach did not inhibit further drinking when the deficit was larger than the capacity of the stomach.

COMMENT. Rates of water ingestion have not been measured in many mammals. Within ten minutes, only a third or half of the water deficit of man was ingested, in deficits of 2 to 3 per cent of the body weight (Adolph, 1937, 1938). The remainder of the deficit required 2 to 5 hours for its removal. The dog is similar to the burro (Adolph and Dill, 1938), which promptly drank very large volumes of water, sufficient at once to remove the complete deficit.

The present measurements reveal close correspondence between the amount of water ingested and the shortage of water in two conditions; namely, in the steady state of the fistulous dog and in the recovery after privation of the normal dog. The normal dog automatically satisfied the water deficit by drinking; ingestion stopped, however, before the water taken had (presumably) gotten beyond the alimentary tract.

The time needed to dispel the urge to drink was exceedingly brief once the passage of water into the stomach had begun, the time being less than 2 minutes when the amount of water required to restore the deficit could be drunk in that time. These and other factors must be taken into account when locating parts of the body that determine when and how much water shall be drunk.

The experiments benefited from the generous help of Dr. R. T. Bellows.

SUMMARY

Water balances were established in dogs so that the daily requirements were kept constant within ± 10 per cent of the mean water intake. The expenditure of water was somewhat reduced when a deficit of water prevailed.

The velocity of water drinking was nearly constant during considerable periods of continuous drinking in the esophageal fistulous dog. The intervals between drafts were also characteristic for each animal.

The rate at which water was drunk (by esophageal fistulous dogs) was approximately proportional to the deficit of water in the body. Sham-drinking was such as would restore the needed water to the body within about 40 minutes for all large deficits.

Real ingestions were exceedingly rapid and proportional, on the average, to the amounts of water needed in the body. The precision with which a dog took an amount of water equal to the deficit was such as to exceed the deficit by about 17 per cent.

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TIME FACTORS IN WATER DRINKING IN DOGS

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Thirst may be defined as the urge to drink water. The satisfaction of thirst is signified by the disappearance of the urge to drink. Much evidence has favored the view that thirst arises only from the local dryness of the mouth resulting from water privation (Cannon, 1933). More recent experiments, however, have indicated that this theory does not suffice to explain the phenomenon of thirst in all the conditions in which it may or may not exist. Dogs have been observed to refuse water although they revealed the external signs of desiccation of dry mouth and loss of skin turgor (Darrow and Yannet, 1935). Thirst may occur when the mouth is wet: marine teleosts have been observed to drink their watery medium (Smith, 1930). Thirst may also appear in the absence of an absolute deficit of water; Gilman (1937) demonstrated thirst in dogs after the intravenous administration of hypertonic solutions. Dogs with diabetes insipidus and esophageal fistulae sham-drank persistently although there was no polyuria or loss of weight (Bellows and Van Wagenen, 1938).

Adolph (1939) has demonstrated that when deprived of water dogs will satisfy their thirst with a single draft of water in 5 minutes or less time, and that in doing so they will ingest accurately the amount of their deficit of water. It may thus be said that in the dog thirst is satisfied by a certain amount of water in the time required to ingest it. In the present investigation three series of observations were made on dogs with esophageal fistulae: *Series 1.* Correlation of the amounts and times of sham-drinking with real drinking. *Series 2.* Investigation of the time element in thirst satisfaction. *Series 3.* The amount and duration of sham-drinking which followed intravenous administration of hypertonic solutions was correlated with the diuresis and rate of solute excretion in the absence of real water ingestion.

PROCEDURE. Two dogs were studied. The management of fistulous dogs has been described previously (Bellows and Van Wagenen, 1938). They were regularly given two feedings daily of equal parts of whole dried milk and "fox chow."

In series 1 and 2 the dogs were made to sham-drink by administering

less than the normal requirement of water. At hourly intervals sham-drinking was permitted for a certain period of time or until the dog refused water for 5 minutes. The amount of water sham-drunk in each successive minute was recorded. In *series 1* a connecting tube, fitted for each dog, made of glass and rubber tubing, was inserted in the fistula to permit real-drinking instead of sham-drinking. This was done 1 hour after the previous sham-drink. In *series 2* the estimated water deficit plus a slight excess was administered by esophagus in 5 minutes. After intervals of variable lengths, sham-drinking was permitted. The administration of water equal to the deficit was timed so that the subsequent opportunity to sham-drink would be 1 hour after the previous sham-drink. In each test the water equal to the deficit was administered 5 to 7 hours after feeding.

Series 3. While in water balance and adipsic the dogs were catheterized, weighed, and bled from the median basilic vein. They were given intravenously 2.5 cc./kgm. of either 20 per cent NaCl or 40 per cent urea. Continuous sham-drinking was permitted immediately and recorded at 5 to 30 minute intervals for 6 or 8 hours and at the end of 24 hours. Urine samples were collected at longer intervals when voided or by catheterization, after each of which the animals were again weighed; 30 minutes after injection another blood sample was taken. Further samples were taken at intervals. At the end of 24 hours stools were collected and the cage was washed.

Chloride determinations were made on serum (Eisenman modification of Van Slyke method, 1929), urine, sham-drunk water, cage washings (Van Slyke, 1923), and stools (Birner, 1928). The urea of the urine, sham-drunk water, and cage washings was determined by the method of Folin and Youngburg (1919); of the serum and stools by the method of Folin and Wu (1919).

The normal feedings were given during the experiments of series 3. Dog B received the first feeding immediately before injection, the second 6 to 8 hours later. Dog C received the first feeding 6 hours after injection, the second 12 hours later. Food chlorides were determined by Birner's (1928) method. Total food nitrogen was calculated from the manufacturer's analysis.

The NaCl injection was repeated in each dog after 1 cc. of pitressin had been given subcutaneously 20 to 30 minutes before injection, and every 2 hours thereafter for 6 hours.

RESULTS. *General.* When allowed to sham-drink intermittently during an unvarying deficit of water the fistulous dog sham-drank approximately the same amount of water every hour when the period of sham-drinking did not exceed 10 minutes. At 30-minute intervals the amount sham-drunk was usually but not always equivalent to the amount sham-drunk at hourly intervals (fig. 1). At intervals of less than 30 minutes the

amount sham-drunk was variable; in general the shorter the interval the less was the amount sham-drunk. At intervals longer than 1 hour the amount sham-drunk did not exceed that at 1-hour intervals. Sham-drinking was constant then when the water deficit was constant and became maximal 30 to 50 minutes after a previous sham-drink.

The manner of sham-drinking was quite uniform in each dog. Dog B sham-drank continuously in one draft with only momentary pauses, after which the animal refused water for 10 or 15 minutes. Dog C sham-drank multiple short drafts of water, the pauses between drafts increasing in duration from 5 seconds to 2 or 3 minutes. Allowed to sham-drink for 30 minutes at 2-hour intervals this dog sham-drank $\frac{1}{2}$ to $\frac{2}{3}$ of the water in the first 10 minutes. Dog B sham-drank continuously twice as much water per minute as did dog C.

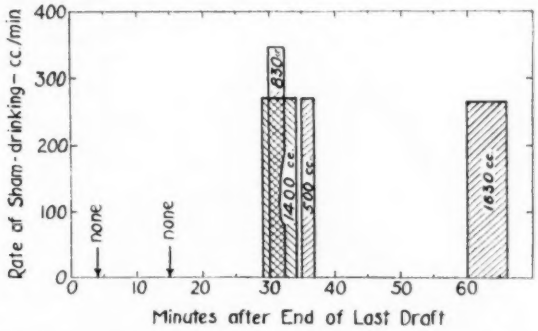

Fig. 1. Total drafts and rates of sham-drinking at intervals in fistulous dog B.

The rate of continuous sham-drinking did not change appreciably as the water deficit increased. Dog B slightly increased the rate of sham-drinking in the largest drafts. Dog C, on the other hand, slightly increased the rate in sham-drinking the smaller drafts (fig. 2). The quantity of water sham-drunk continuously at hourly intervals regularly increased as the deficit of water increased (fig. 3). Variations in the amount of sham-drinking from hour to hour appeared to be related to the feedings (fig. 3). When water privation was moderate, sham-drinking temporarily decreased after feeding (day 2). In more severe privation feedings had little effect (day 3), or temporarily increased sham-drinking (day 4, 4:00 p.m.).

The observations on dog B illustrate with especial clarity that *a*, sham-drinking was proportional to the water deficit, and that *b*, it apparently served to satisfy thirst temporarily.

Series 1. After the connecting tube was inserted the dogs were able to ingest the water they drank. This artificial restoration of the continuity

of the esophagus proved as satisfactory as the natural esophagus in water drinking, for the dogs invariably terminated their privation when permitted to drink with the connecting tube (fig. 3). Water was drunk by dog B

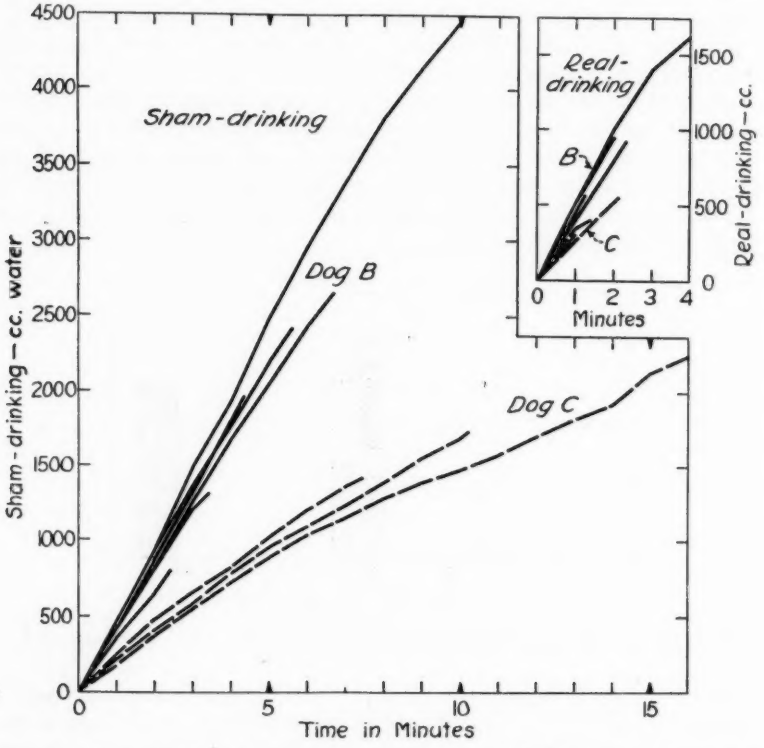

Fig. 2. Sham-drinking in two dogs with esophageal fistulae. The observations on dog B were all made at hourly intervals; each line represents 7 drafts sham-drunk continuously, with the exception of the longest line which represents 2 drafts. The observations on dog C were made at 2-hourly intervals; each draft was sham-drunk interruptedly, the time not spent in sham-drinking being subtracted; the total time allowed for sham-drinking was 30 minutes. Each of the shorter lines is the average of 5 drafts; the longest line is the average of 4 drafts.

Real-drinking with connecting tube in the same two dogs. Dog B drank continuously. Dog C drank with short interruptions, the total time spent in drinking not exceeding 5 minutes.

with the tube at the same continuous rate as it was sham-drunk. When large quantities were drunk there was a moderate diminution in the rate toward the end of the draft, which was not noted in sham-drinking (fig. 2).

Dog C drank about twice as fast with the tube as without (fig. 2). It is possible that the sham-drinking of this animal was impeded by the aspiration of air through the upper fistulous opening. Both animals completed their draft of real-drinking within 2 to 4 minutes. That they had correctly removed their deficit was affirmed by their refusal to sham-drink 1 hour later as well as at subsequent periods (fig. 3).

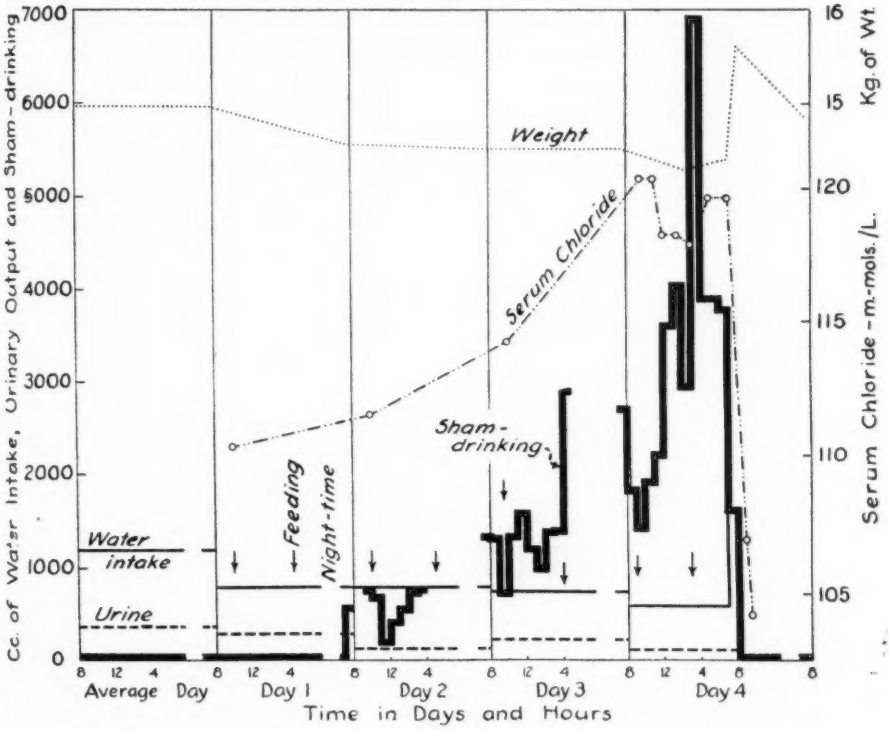

Fig. 3. Sham-drinking by dog B during progressive desiccation, and correlation of sham-drinking with real-drinking. The water intake and urine output are recorded as cubic centimeters per day. Sham-drinking is recorded as cubic centimeters taken in 1 draft each hour. At 8:00 p.m. on day 4, 1 hour after last sham-drink, connecting tube was inserted and the dog allowed to real-drink.

The amount sham-drunk in the last one or two of a series of hourly sham-drinking periods during increasing privation bore a definite relationship to the amount subsequently drunk with the connecting tube. In table 1 are shown the correlations in several such tests. The water deficit of these dogs could thus be computed from the amount of water

sham-drunk at hourly periods; dog B sham-drank in one draft approximately 250 per cent of the deficit; dog C sham-drank in 10 minutes 155 per cent to 170 per cent of the deficit. Thus to the three bases of computing water deficit described by Adolph (1939) may be added basis 4 ("sham-drinking deficit").

During the greatest deficit (11 per cent of body weight) developed in dog B the animal was irritable and the mucous membranes were dry and sticky. The irritability was but briefly allayed by sham-drinking. Following the real-drinking the animal became permanently quiet and relaxed.

TABLE 1

Correlation of continuous sham-drinking with real-drinking

DOG	TEST	TIME	SHAM-DRINK	REAL-DRINK	PER CENT OF REAL-DRINK	PER CENT OF WEIGHT
			cc.	cc.		
B	1	3 hours before	3,920		240	
		2 hours before	3,925		240	
		1 hour before	3,800		232	
				1635		11
		1 hour after	0			
B	2	2 hours before	2,100		220	
		1 hour before	2,475		256	
				960		6
		1 hour after	0			
C	1	4 hours before	830		158	
		2 hours before	900		171	
				525		3
		1 hour after	0			

Series 2. When sham-drinking was permitted after administration of the estimated water deficit by esophagus, the results varied according to the length of the interval between the completion of administration and the beginning of sham-drinking. Figure 4 (top) shows the details of one experiment in dog B. The water deficit was computed from the amount sham-drunk in the last of a series of hourly sham-drinks. Figure 4 (bottom) shows the composite of this series of experiments. From the moment the administration was completed until the end of 9 minutes the dog promptly sham-drank in one draft, *not the amount previously sham-drunk, but only the amount of the deficit.* From the 10th to the 15th minutes inclusively there was an abrupt change in the amount and manner of sham-drinking; the dog sham-drank only small amounts of water or about 20 per cent of the deficit. This sham-drinking was frequently

started after a delay and progressed in drafts interspersed with pauses as long as 20 seconds. From the 16th to the 30th minutes inclusively the dog refused to sham-drink. After each of the experiments the dog refused to sham-drink one hour later.

This series of experiments, in which water is ingested before it is drunk, reveals the operation of at least two factors in the satisfying of thirst: 1, an immediately acting factor above the fistula, and 2, a factor below

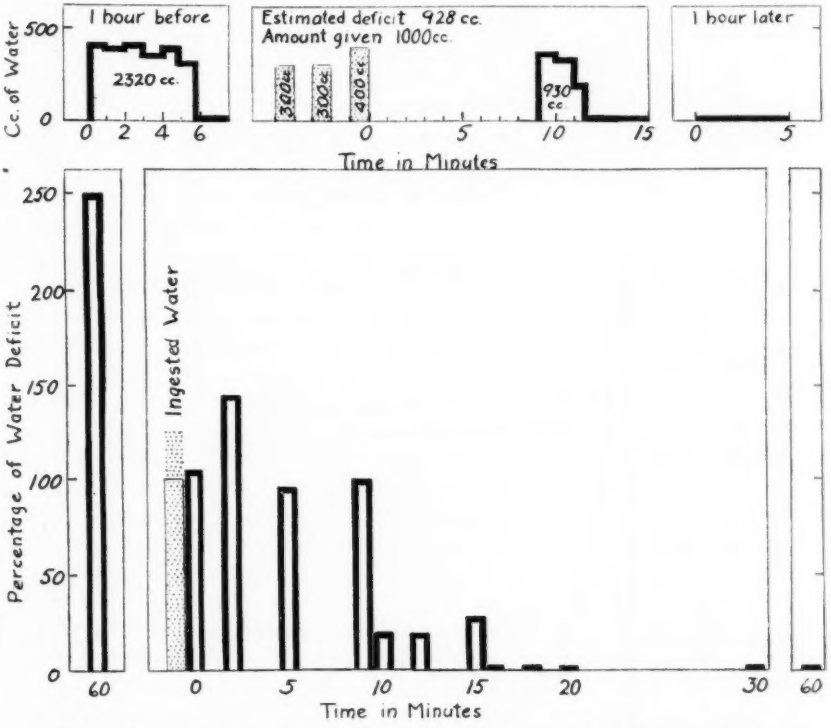

Fig. 4. Sham-drinking at various intervals after administration by esophagus of the calculated deficit of water in dog B. *Top*, detail of one experiment. *Bottom*, summary of similar experiments. The heavily outlined blocks represent sham-drinking, the stippled blocks represent administered water.

the fistula acting after a delay. It further demonstrates that the subfistulous factor has an immediate inhibiting influence on the repetitive act of drinking.

Series 3. Sham-drinking after the intravenous injection of hypertonic solutions varied according to the solution employed. The results were similar in each dog for each solution. The detailed record of the NaCl

experiment in dog B is shown in figure 5. Injections required about 1 minute.

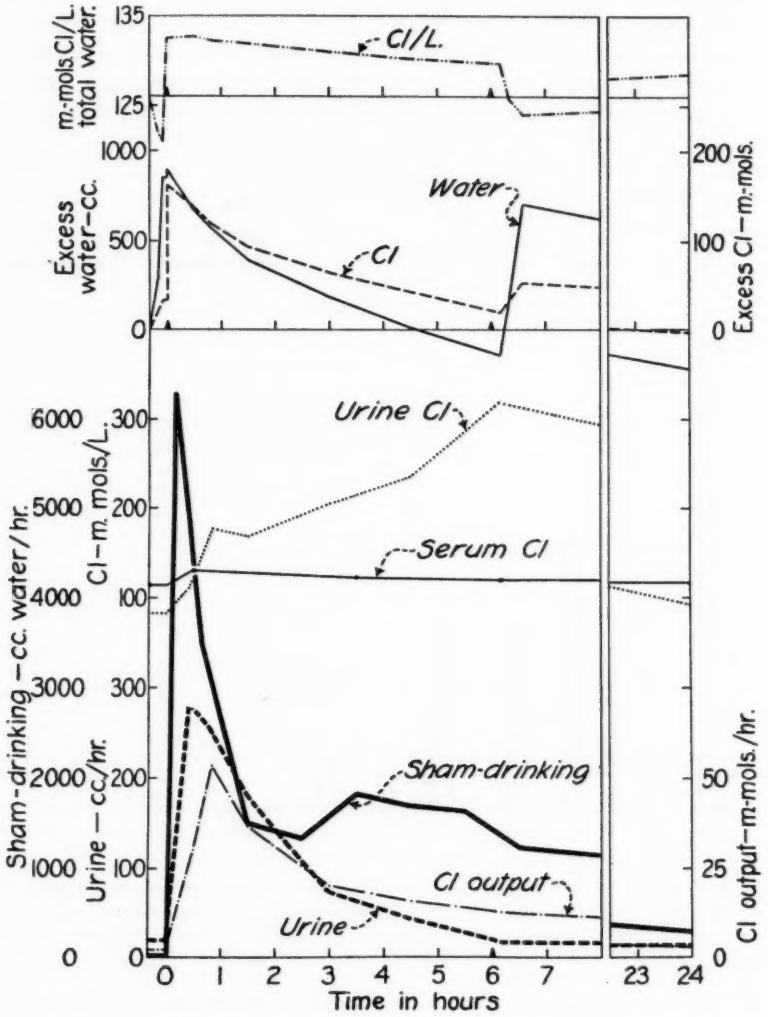

Fig. 5. Observations on water and Cl exchanges after the intravenous injection of 2.5 cc. of 20 per cent NaCl solution per kilogram. The upper curves are calculations of the water and Cl balances and of the ratio of total Cl to total body water.

During injection of 20 per cent NaCl the dogs began to lick their lips. They started to sham-drink as soon as they were returned to the cage

about 1 minute after completion of the injection. The subsequent discontinuous sham-drinking reached its maximal rate 10 minutes after injection, preceding the maximal diuresis by about 15 minutes. It then rapidly declined to a low rate which persisted until the dog was fed at the end of 6 hours. Dog B continued to sham-drink at a low average rate during the night; dog C did not sham-drink after the 6-hour feeding.

The curves of sham-drinking of both dogs were surprisingly alike in spite of the fact that dog C, which was not fed before injection, developed a weight deficit almost at once, whereas dog B had a weight excess for 4 hours. The 6-hour feeding removed the weight deficit in both cases. Dog B ended 24 hours with a deficit of 215 grams; dog C, having received the second feeding at 12 hours, ended the day in weight balance. All the chloride injected as well as the food chloride was fully eliminated by both animals in 24 hours. Thus water deficits computed on the basis of weights were not important factors in sham-drinking.

The ratio of total Cl to total body water of dog B was computed from the data of Harrison, Darrow and Yannett (1936). The Cl: total water ratio was increased from the normal of 126.0 m.-mols. per liter to 132.6 m.-mols. per liter by injection. It gradually declined in 6 hours to 129.6 m.-mols. per liter (fig. 5). The ratio was reduced by the feeding but had again risen above normal by the following morning.

Since no observations were made on serum Cl within the first half-hour after injection, it is of interest to compute what the maximal concentration of plasma Cl would have been at the end of injection. Assuming the plasma to have been 5 per cent of the weight of 14.96 kgm. there were 748 cc. of plasma. Adding 128.1 m.-mols. of Cl injected to the plasma already containing 114 m.-mols. per liter, there would have been 285.2 m.-mols. of Cl per liter of plasma at the completion of injection had none left the blood stream. Although the time of injection was only 1 minute it is probable that Cl was diffusing out of the plasma during this time and that the plasma never attained this concentration. But it is also probable that the maximal concentration was considerably higher than 130.2 m.-mols. per liter of plasma, which was observed ½ hour later. Moreover, the precise onset and maximal rate of sham-drinking coincided with this high serum Cl concentration.

Sham-drinking which followed 40 per cent urea differed from that after NaCl in two important respects: 1, it did not begin at once but only after a delay of 10 to 15 minutes; 2, its curve was a miniature replica of that after NaCl. The maximal rate developed within 5 or 10 minutes after sham-drinking started and then subsided within an hour to an average rate one-quarter to one-third that of NaCl for the next 5 hours. The maximal rate was approximately 50 per cent of the maximal rate after NaCl. There was no further sham-drinking after the 6-hour feeding.

Diuresis was also less marked. In dog B maximal diuresis preceded

maximal sham-drinking; in dog C maximal diuresis did not occur until 1½ hours later.

The only significant respect in which pitressin affected sham-drinking after NaCl injection was in causing *a delay of 10 to 20 minutes* before sham-drinking started.

Discussion. There are at least two factors concerned in the satisfaction of thirst.

1. *Buccal and pharyngeal factor.* Sham-drinking demonstrates that the passage of water through the mouth and pharynx confers temporary satisfaction of thirst. The amount of water required to do this exceeds but is proportional to the deficit. Several processes are therein concerned. Cannon (1933) and Gregersen (1938) believe satisfaction is secured by wetting the mucous membranes of the mouth and pharynx. Wetting, however, should be as effectively obtained by sham-drinking as by real-drinking, since the rate of drinking is the same in each case. Each gulp of water causes distention of the pharynx before it enters the esophagus. Some human individuals localize their sense of gratification as occurring in the pharynx. The repetitive act of drinking is accomplished by muscular movements of the lips, tongue, pharynx, and respiratory apparatus, which aspirate and propel the water along its course. This repetition of muscular movements is suggested as a possible factor by which satisfaction is secured. Satisfaction is obtained in many instinctive desires by muscular movements. Furthermore, the drinking of fluid constitutes the only condition in which swallowing may be rapidly repeated.

2. *The subpharyngeal factor.* This factor may consist of one or more processes. Entrance of water to the amount of the deficit into the alimentary tract below the pharynx inhibits the repetitive act of drinking to the actual amount of the deficit. This factor also confers permanent satisfaction of thirst after a delay of 10 to 15 minutes. This is shorter than the complete absorption time, for Klisiecki et al. (1933), and others, have determined that the complete absorption of ingested water requires 35 minutes. It is appreciated, however, that the permanency of the satisfaction of this factor may depend on the ultimate absorption of the deficit.

The immediate inhibitory effect of this factor on drinking suggests the operation of mechanical or nervous processes, which might be obtained by the dilatation of parts of the alimentary tube by the water. The permanent but delayed complete satisfaction of thirst may possibly be obtained through the intervention of a pituitary hormone factor.

The sudden appearance of intense thirst during or immediately following the injection of hypertonic NaCl solution is in marked contrast to the gradual development of thirst from water privation. It was the direct result of the addition of NaCl to the body and occurred before the NaCl caused diuresis. Gilman (1937) has suggested that cellular dehydration after NaCl is the stimulus of thirst. While this explanation seems logical for the sustained thirst which occurred later, another factor seems to be

concerned in the production of the immediate maximal thirst. Its suppression by pitressin suggests a relationship with the pituitary hypothalamic mechanism, injury of which in diabetes insipidus also causes thirst.

SUMMARY

Dogs with esophageal fistulae were allowed to sham-drink at regular intervals during water privation. The quantities of water sham-drunk were proportional to the water deficit, and provided a basis for computing the deficit. Observations were made on sham-drinking after a quantity of water equal to the deficit had been administered by fistula.

The satisfaction of thirst was found to be not a single process, such as wetting the mucous membranes of the mouth, but a series of at least two supplementary and consecutive processes. Passage of an excessive amount of water through the mouth and pharynx confers immediate but temporary satisfaction of thirst. The repetitive act of drinking and swallowing water is inhibited when the ingested water deficit enters the gut below the upper esophagus. The short period of temporary satisfaction secured by the passage of water through the mouth and pharynx is superseded by the delayed process of permanent satisfaction operating lower in the gut.

Sham-drinking was allowed *ad libitum* after the intravenous injection of hypertonic solutions of NaCl or urea. After NaCl, sham-drinking started at once and reached a maximum in 10 minutes, preceding the maximal diuresis. After urea, sham-drinking started after a delay of 10 to 15 minutes. Pitressin administration before NaCl injection inhibited sham-drinking for 10 to 20 minutes.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF HEPARIN IN THE DOG[1]

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The remarkable potency of heparin and the fact that it can be used in vivo led Howell (1924) to suggest that it should be of great value for both laboratory and clinical purposes. The work which has been done in this laboratory along these lines has been reported elsewhere (Murray, Jaques, Perrett and Best, 1937). Before these investigations were begun it was necessary to study the physiological effects of injections of the anticoagulant. The results of these studies are reported here. Two methods were adopted for the purpose. The continued presence of the injected heparin in the blood stream was followed by means of the resulting increased clotting time: the heparin content of tissues and body fluids before and after injection was determined by the method of Charles and Scott (1933).

By means of the effect on the clotting time, the dosage of heparin can be controlled. Various workers (originally Howell) have reported that, on injection, heparin causes a very prolonged clotting time and that this effect rapidly wears off, the duration of the effect depending on the dosage. While the time relationships for a single dose have been established, there has been no attempt to find any general relationship between dosage and the effect on clotting time although Howell has drawn attention to the importance of such a study.

The cause of the rapid disappearance of the heparin effect has been studied even less, although it has generally been assumed (Pupini, 1932) that it is due to excretion of the heparin as suggested by Howell and MacDonald (1930). We have not observed any excretion of injected heparin. Fuchs (1933) and Sato (1932) suggested that the liver might be involved in the removal either by storing or inactivating the injected heparin. We have not observed any storage in the liver so the actual fate of injected heparin is unknown.

EXPERIMENTAL METHODS. Normal dogs of 8 to 15 kgm. body weight were used. Amytal or nembutal was used when an anesthetic was necessary. Connaught Laboratories' 15-unit heparin was used in the early

[1] Read in part before the Royal Society of Canada, May 1935.

experiments and was dissolved in physiological saline before use. In the later experiments the 100-unit material prepared from the crystalline barium salt of Charles and Scott (1936) was used and was supplied as a solution in saline of 1000 u./cc.[2] Control experiments on the same animal using the two preparations have not shown any physiological difference between them except the complete freedom from toxicity of the 100-unit material (see Murray, Jaques, Perrett and Best, 1937). In all cases the heparin was injected into the radial or femoral vein of a limb not used for obtaining blood samples.

In studying the effect of heparin injections, the determination of the concentration of heparin in the blood by means of the clotting time produced is convenient and rapid but has the disadvantage that only a small

TABLE 1

UNITS OF HEPARIN	CLOTTING TIME*
0.00	1 min. 50 sec.
0.23	2 min. 13 sec.
0.45	4 min. 15 sec.
0.60	7 min.
0.75	8 min. 15 sec.
1.5	15 min.
2.25	26 min.
3.0	Incoagulable

* Clotting times by method of Lee and White.

Heparin 15 u./mgm. was used. Two blood samples from each of four dogs were taken for each dilution and the eight values thus obtained were averaged.

TABLE 2

EXPERIMENT	DOG	HEPARIN	DURATION OF HYPOCOAGULABILITY	HEPARIN DURATION
		u./kgm.	*minutes*	
6	B	30.7	27	1.2
8	A	113	73	1.6
9	A	198	91	2.9
10	B	334	98	3.4
12	A	420	200	2.1
13	B	503	234	2.1
14	D	341	180	1.9
15a	D	556	200	2.8
15b	B	667	310	2.2
Average.....				2.2

Clotting times by method of Lee and White.

Heparin 15 u./mgm.

range of heparin concentrations can be measured. It was found, however, that the clotting time obtained with a given amount of heparin varies considerably with the method of measurement. For this reason it was feasible to adopt two methods which measured different ranges of concentration of heparin in the blood. With the method of Lee and White (1913) (8 mm. tubes at 20°C.) with blood samples drawn from the saphenous vein through the skin, heparin concentrations from 0.5 to 4.0 units per cubic centimeter of blood could be measured. A lower range (0.05 to 0.4 u./cc.) could be

[2] The unit used in this paper is that of the Connaught Laboratories and has been found to be equal to the potency of $\frac{1}{160}$ mgm. of Charles and Scott's crystalline barium salt. The potency of this unit is the same, within the limits of assay, as that of Hynson, Westcott and Dunning based on the original Howell unit.

measured in the coagulometer previously described (Murray, Jaques, Perrett and Best, 1937) using blood carefully drawn from the exposed femoral vein. The methods were standardized by adding 1 cc. samples of blood, drawn in exactly the same manner as in the experiments, to 0.2 cc. of saline containing varying amounts of heparin. Table 1 shows the standardization curve obtained for the method of Lee and White. With the second method (for low heparin concentrations) great variation was found among different animals. A standardization curve was therefore established for each dog studied and several typical results are shown in figure 1. These standardization curves were then used to convert the clotting times

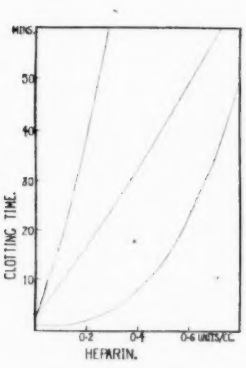

Fig. 1

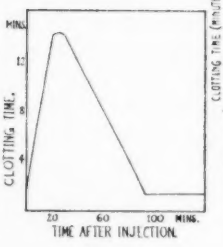

Fig. 2

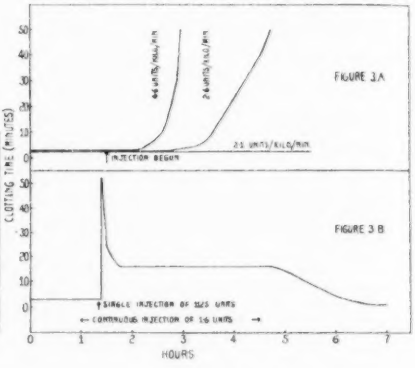

Fig. 3

Fig. 1. Relationship between heparin concentration and clotting time in dogs. Clotting time by the coagulometer.

Fig. 2. The effect of a single intravenous injection of heparin; 2200 units given to an 11 kgm. dog. Clotting times by the method of Lee and White.

Fig. 3. The effect of a constant intravenous injection of heparin on the clotting time. 3B. Constant injection of 1.6 u./kgm./hr. + a single injection of 1125 units. Clotting times by the method of Lee and White.

found to units of heparin per cubic centimeter. Crafoord (1937) has studied this in vitro-heparin-clotting time curve using blood from human patients and has suggested its application clinically. The simplest treatment of the curve, as he suggests, is to draw it as two straight lines. The data obtained in the experiments reported showed best agreement with an equation of the form $T = C\,[1 + (Ax)^p]$ where T = clotting time, C = normal clotting time, A and p are constants and x = heparin concentration. This is the same as that found by Barratt (1937) for a heparin-plasma-thrombin system. Heparin concentration in the blood refers only to the concentration of added heparin and does not include the normal heparin content of dog's blood reported in table 4.

The heparin content of the tissues of dogs which had received large doses of heparin, and also of normal dogs, was assayed according to the method of Charles and Scott (1933). I am indebted to Doctor Charles for his advice and assistance in these assays. The "crude heparin" obtained from the purification process was assayed against a standard heparin preparation using cat's blood. After the necessary approximate dilution had been determined by preliminary assay tests, three tests were made. Where there was a great spread in these values, two to four more tests were made and all the values averaged. Feces were treated in the same manner as the tissues. Urine was concentrated to half volume in vacuo at 50°C. and precipitated with two volumes of acid alcohol. The precipitate was dissolved, precipitated with acid acetone, washed, dried and assayed. In control experiments with added heparin, about 70 per cent of the added heparin was recovered.

THE EFFECT OF INJECTIONS OF HEPARIN ON THE CLOTTING TIME. Subcutaneous injections of heparin are effective in small animals like the rabbit but have little effect in the dog unless the dose is very large. Hence the intravenous method has been used exclusively.

The effect of a single injection of heparin. As reported by a number of observers (originally by Howell), when heparin is injected intravenously, there is a rapid rise in the clotting time followed by a more gradual but still rapid descent to the normal. An example of this is shown in figure 2. An 11 kgm. dog was given 2200 units and it can be observed that the clotting time rose to a maximum of 15 minutes and then rapidly returned to normal. From such an experiment two values can be derived, namely, the maximum clotting time and the duration of the heparin effect.

The maximum clotting time obtained depends on the heparin dosage. Provided that the potency of heparin is the same in vivo as in vitro, the maximum clotting time should correspond with that obtained on adding an equivalent amount of heparin to a normal sample of blood after withdrawal. Good agreement has always been found in our experiments. As was seen with the in vitro standardization curve, with high concentrations of heparin the resulting clotting times were approximately the same with the same doses in different animals but with low concentrations there was considerable variation; i.e., the maximum clotting time following the same dose of heparin showed great variations with the different dogs but in all cases agreed with that found in vitro for the same animal. The fact that considerable time (about 10 minutes) elapses before the peak of the clotting time curve is reached is difficult to explain. Quick (1936), however, has observed a similar phenomenon in vitro.

The duration of the hypocoagulable condition of the blood (due to the presence of heparin) must evidently depend on the rate of disappearance of heparin from the blood stream. In table 2 is shown the duration of the

effect for a series of single large doses of heparin. It can be seen that the duration of the effect is directly proportional to the heparin dosage as the ratio of the two is a constant. A possible explanation of this fact might be that the rate of removal of the heparin from the blood is constant. This could be determined directly by measuring the slope of the original curve. In the case of figure 2 the slope was found to be 0.20 (i.e., 2.0 u./kgm./min. compared with the average of 2.2 u. shown in table 2). A better and more decisive way of testing the same point is to study the effect of continuous injections of the anticoagulant. A large number of experiments of this nature were therefore carried out. Studies were conducted with both clotting time methods, measuring (1) high heparin concentrations and (2) low heparin concentrations in the blood. The results will be discussed separately.

The effect of continuous injections of heparin. The results of a large series of experiments using the Lee and White method are illustrated in figure 3 by several typical experiments. When the rate of injection was greater than 2.0 u./kgm./min., the clotting time rose until it became immeasurable. With a lower rate of injection, the clotting time remained unchanged. This shows that the rate of disappearance was constant (2.0 u./kgm./min.). A series of experiments were then conducted in which the effect of combining a single injection with the continuous injection was studied (fig. 3 B). A continuous injection of 1.6 u./kgm./min. (this having been previously found to be the rate of removal for the dog used) was established and was followed by a single injection of 1125 units (1.6 units per cc. of blood which should give a clotting time of 16 minutes). The clotting time, after an initial rise, remained at an average value of 16 minutes for 2½ hours at which time the injection was discontinued. These results suggest that the rate of disappearance of heparin from the blood of the dog is relatively constant with large doses of the anticoagulant.

In another large series of experiments the coagulometer was used to measure lower heparin concentrations in the blood and in order to be able to measure the clotting times, the rate of injection was less than 1 u./kgm./min. Under these conditions, it was found that the clotting time rose gradually to a new level, at which it remained constant. Examples are shown in figure 4. As this level means that the rate of disappearance of the heparin was exactly equal to the rate of injection, it gives the rate of disappearance of the heparin from the circulation. By means of the in vitro standardization curve for the animal, it is possible to convert the clotting time level to units per cubic centimeter of blood. This was done for a number of experiments and the resulting heparin content of the blood was plotted against the rate of injection (fig. 5). A linear relationship can be observed between the heparin level obtained and the rate of injection (rate of disappearance). These results mean that with low heparin concen-

trations the rate of removal of heparin is proportional to the heparin concentration in the blood. The close agreement between the results obtained in different animals is surprising, as with the same rate of injection the clotting time level might be 7 minutes in one dog and 30 minutes in another.

Figure 4 also illustrates the effect of a single injection along with the continuous injection. The single injection causes an immediate rise in the clotting time which then gradually falls to the level found with the continuous injection alone. The result is similar to that shown in figure 3 B for much larger injections administered in the same way. This is the most

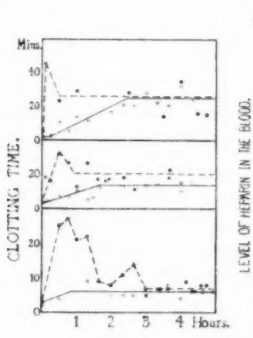

Fig. 4

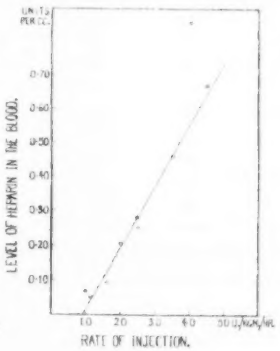

Fig. 5

Fig. 4. The effect of a constant injection of heparin. ○—○--○ Continuous injection alone. ●—●—● Continuous injection + a single injection of 35 u./kgm. Continuous injection of 25 u./kgm./hr. in the two upper figures, 11.4 u./kgm./hr. in the lower one. Clotting times by the coagulometer. In each experiment the continuous injection was begun and the single injection given at zero ti me on the graph.

Fig. 5. The level of heparin in the blood established by a constant injection of the anticoagulant.

practical method of administering heparin for most purposes (see Jaques, Charles and Best, 1938).

The heparin content of the tissues of the dog (normal and heparinized). It was seen above that heparin is rapidly removed from the circulation. In an attempt to throw some light on the site of this process, various tissues of the dogs were assayed for their heparin content. The association of heparin with liver (Howell) and with lung (Charles and Scott) led us to assay these tissues first. As no difference was found in their heparin content after the injection of large amounts of the anticoagulant, the study was extended to other tissues and to the excreta. Table 3 shows the heparin content of the excreta of a dog for a week before and after the injection of a single dose of 4500 units of heparin. It can be seen that urine and

TABLE 3

Heparin content of excreta

URINE			FECES		
Date, 1935	Units per day	Urine volume	Date, 1935	Weight	Units
		cc.		*grams*	
March 26	55	150	March 25 to April 1	180	11
27	50	70			
28	50	75			
29	32	83			
30	32	122			
31	33	90			
April 1	33	90			
April 2	60	460	April 1 to 4	116	11
3	50	330	April 4 to 6	148	15
4	30	200			
5	25	270			
6	60	224			

On April 1 the dog was given an injection of 4500 units in 5 cc. of saline intravenously. The urine was collected in the morning of the day given.

TABLE 4

Heparin content of tissues

(units/100 grams fresh tissue)

	NORMAL DOGS			HEPARINIZED DOGS		
	X	R	Average	U	S	W
Units of heparin received				13,500	7,500	3,000
Lung	1,200	1,000	1,100		830	750
Liver	2,840	2,750	2,800	3,030	2,800	520
Spleen	260	160	210	68	500	
Heart	49	156	100	240		159
Kidney	378	450	400	500	250	100
Muscle	500	340	400	230	160	150
Blood		11			3	
Gut	740	724	730	1,080	1,440	900

Dog S received a single injection of 7500 units of heparin and was sacrificed 8 hours later when the clotting time had returned to normal. Dog U had received 13,500 units during the previous month and died under amytal anesthesia from cardiac failure. Dog W received 3000 units 2 weeks before death, the cause of death being pneumonia. The assay figures are expressed as units per 100 grams of fresh tissue. In only 2 dogs was there sufficient heparin in the blood to get an assay figure.

Doctor Charles kindly assayed the antiheparin (kinase) content of the blood of dogs R and S and of the urine samples for dog S (table 4) by the method of Charles, Fisher and Scott (1934). No antiheparin was found in urine and no increase of this substance in blood after injection could be observed.

feces normally contain small amounts of an anticoagulant but that the injection of heparin does not lead to any increase in the amount of this substance. Table 4 shows the heparin content of various soft tissues of five dogs (two normal and three heparinized). No significant increase can be observed in the heparin content of lung, liver, kidney, muscle or heart after injection. There was an increase in the heparin content of spleen but only in the dog killed on the same day as the injection, suggesting that the extra heparin might be present in the blood of that organ. In all three dogs which had received heparin there was a definite increase in the heparin content of gut. In the case of dog S, 1300 units out of 7500 units injected were recovered from this tissue.

Discussion. From these results it appears that the rate of disappearance of heparin from the blood stream is proportional to the concentration of heparin in the blood. With increasing concentrations, however, a limiting value is reached beyond which a further increase in concentration does not cause any increase in the rate of removal. The limiting rate of removal is about 2 u./kgm./min. The fact that the rate of disappearance for a given concentration of heparin in the blood shows only slight variations in different dogs means that the duration of the heparin effect will show little variation—an important point in the administration of the anticoagulant. The effect produced, i.e., the clotting time obtained, will depend on the relative activity of the various clotting factors in the blood and hence shows considerable variation among individual animals. As, however, there is no difference in the potency of heparin in vivo or in vitro, as judged by the maximum clotting time, it is possible to determine the effect of a given dose in an individual animal before injection by the addition of the equivalent amount of heparin to the blood in vitro.

These findings can be applied in the administration of heparin as it is possible to calculate, from the values given, the heparin dosage necessary 1, to obtain a definite concentration of heparin in the blood, or 2, to obtain a desired clotting time. Similarly, it is possible to calculate the heparin concentration and clotting time resulting from a given dose. The rate of injection necessary to give a desired concentration of heparin in the blood is shown in figure 5. To obtain any desired clotting time it is necessary to find the heparin concentration necessary to give the desired clotting time in the individual animal, i.e., to establish the in vitro standardization curve (clotting time-heparin concentration) and then make use of figure 5 to obtain the necessary rate of injection. When a very high concentration of heparin in the blood is desired, a procedure similar to that in figure 3 B must be followed, but such high concentrations do not appear necessary for most purposes. The above procedure has been applied in these laboratories and has been checked by means of the reaction of heparin with protamine (Jaques, Charles and Best).

It is evident that heparin when injected does not appear in the urine and feces but that the injection does lead to an increase in the heparin content of gut. The significance of this latter finding is at present unknown. The only direct experiments on this problem known to us are those of Iankovskii (1930) who studied the effect of removal of various viscera on the duration of heparin action. His results also indicate that the gastro-intestinal tract is involved in the removal of heparin from the circulation. The values reported for the heparin content of tissues of normal dogs are of interest. These values are much higher (3 to 10 times) than those obtained by Charles and Scott for the corresponding tissues of the ox. Further, it is of interest that dog liver contains more than twice as much heparin as dog lung, whereas ox lung contains more heparin than ox liver (Charles and Scott, 1933). Dog liver has much the highest heparin content of any tissue thus far assayed. The fact that gut contains large amounts of the anticoagulant is also of interest.

SUMMARY

1. The relationship between heparin concentration and clotting time is $T = C\,[1 + (Ax)^p]$ where T = clotting time, C = normal clotting time, A and p are constants and x = heparin concentration. The potency of heparin is essentially the same in vitro and in vivo so that the clotting time for a given dose of heparin administered intravenously can be found from the standardization curve in vitro.

2. The duration of the heparin effect depends on the rate of removal of heparin from the circulation. When the heparin concentration in the blood is 1 unit per cubic centimeter or less, the rate of removal is proportional to the concentration. With concentrations of 2 units per cubic centimeter or more, the rate of removal is constant (2 u./kgm./min.).

3. The application of these findings in the administration of heparin is discussed.

4. The injected heparin does not appear in the feces or urine.

5. Injection of heparin does not lead to an increase in the heparin content of any tissue except intestine.

6. The normal heparin content of various tissues of the dog has been determined and, expressed in units per 100 grams of fresh tissue, is as follows: lung 1100, liver 2800, spleen 210, heart 100, kidney 400, muscle 400, blood 10, intestine 730. The significance of the finding that intestine contains relatively large amounts of heparin is not known at the present time.

The author wishes to acknowledge his indebtedness to Prof. C. H. Best and Dr. D. W. G. Murray under whose direction this work was carried out.

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THE MECHANISM OF THE ACTION OF SALIVA IN BLOOD COAGULATION

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It is well known that the addition of saliva to blood will accelerate its coagulation (1, 2, 7). This property of saliva is not species specific. It has been shown that dog, cat and human saliva will indiscriminately accelerate the clotting of blood obtained from the dog, cat, cow or man (1, 7). Bellis, Birnbaum and Scott (1) noted that while the clotting factor of saliva was stable at 60°, it was destroyed by boiling at 100°. Bellis and Scott (2) found that the addition of saliva decreased the coagulation time of hemophilic blood, and that a similar effect was obtained with peritoneal and cerebrospinal fluids.

The present investigation was undertaken to determine the function of saliva in the coagulation mechanism and to investigate the nature of the active material. The results obtained indicate that saliva contains a factor which acts like the thromboplastic material present in blood platelets, brain and lung tissues and that it probably is protein in nature.

EXPERIMENTAL. The reagents employed in this investigation were prepared as follows: *Plasma*—from beef blood obtained by the addition of 0.2 per cent potassium oxalate. *Prothrombin*—prepared according to the method of Mellanby (8). The product was not activated by calcium ions alone when incubated for the maximum length of time of the experiments. *Fibrinogen*—obtained by repeated precipitation with sodium chloride as described by Eagle (3). *Saliva*—human saliva was collected by first rinsing the mouth with saline and the chewing of paraffin to stimulate the flow. Samples were used within a few hours of collection. *Calcium chloride*—0.025M solution. *Thromboplastin*—a fresh saline extract of blood-free calf-brain.

The clotting mechanism may be separated into two well-recognized stages; 1, the formation of thrombin, and 2, the formation of fibrin from fibrinogen. The first step in analyzing the rôle of saliva was to determine which of these two stages was influenced by the presence of saliva. The following observations show that saliva has no important action on fibrinogen but that it is active in the formation of thrombin.

When fresh, filtered saliva is added to purified fibrinogen, no clot

forms. After several hours' standing, a few strands of a fibrin-like material do appear. However if the saliva is boiled for a few minutes before being added to the fibrinogen, no fibrin is formed. Since the heated saliva is still active in accelerating the coagulation of blood as is shown later, the action of saliva must be on the formation of thrombin.

The slight action on fibrinogen is probably due to the presence of proteolytic enzymes in saliva (4) which are destroyed on heating.

The formation of thrombin is dependent on a reaction involving prothrombin, ionic calcium and a thromboplastic substance such as cephalin or certain tissue extracts. The amount of thrombin which has been formed may be approximately measured by adding the reaction mixture to a solution of purified fibrinogen in physiological saline, and measuring the coagulation time. The coagulation time has been found to be directly proportional to the amount of thrombin present (9).

Using this method it can be demonstrated that saliva cannot take the place of prothrombin or calcium ions. It can replace the thromboplastin of brain. The obvious conclusion to be drawn from these experiments is that saliva contains a thromboplastic material.

Thromboplastic activity may be assayed by the following method similar to the one reported by Ferguson (6) for cephalin.

A mixture of 0.7 ml. of prothrombin solution, 0.2 ml. calcium chloride solution and 0.1 ml. of thromboplastic material is incubated in a water bath at 38°. After definite intervals of time (marked *incubation time* in the figures), 0.1 ml. portions are pipetted off and added to 1.0 ml. of fibrinogen solution or to plasma (diluted 1:4 with saline to remove the effects of anti-thrombic substances which are present) in 10 by 75 mm. serological tubes maintained at 38°. The *coagulation time* is measured by tilting the tubes every 5 seconds until the mixture ceases to flow. The elapsed time is measured from the addition of the thrombic mixture with a stopwatch.

Plotting the coagulation time against the incubation time gives a series of curves which represent the relative amounts of thrombin formed at the different intervals. The prothrombin and fibrinogen content may differ considerably from batch to batch, but for any given experiment the properties of these materials are constant. The main controlling variable is therefore the concentration of thromboplastin, which markedly affects the rate of thrombin formation and the amount of thrombin formed. If a standard thromboplastin preparation is used, such as Ferguson's cephalin (5) kept under absolute alcohol, the above method may be used for the quantitative assay of thromboplastic activity. Curves illustrating the experimental procedures carried out on the blood coagulation accelerating properties of saliva in which coagulation time is plotted against incubation time, are given in figures 1 to 4. Because of the variation in

prothrombin and fibrinogen content, a curve representing the effect of whole or supernatant saliva, on each batch of prothrombin and fibrinogen (to serve as a control), is given in each of the figures representing the results of an experimental series.

It has been found that the thromboplastic material of saliva is about as effective as the thromboplastin of brain in promoting the formation of thrombin, if it is sufficiently concentrated. This is brought out by curves *2* and *3* of figure 1, which gives a comparison of a suspension of sediment from saliva with that of a fresh saline extract of calf brain.

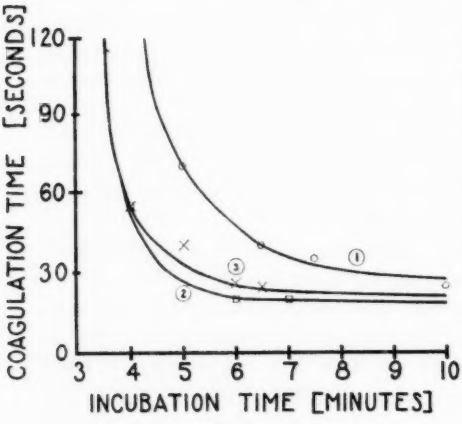

Fig. 1

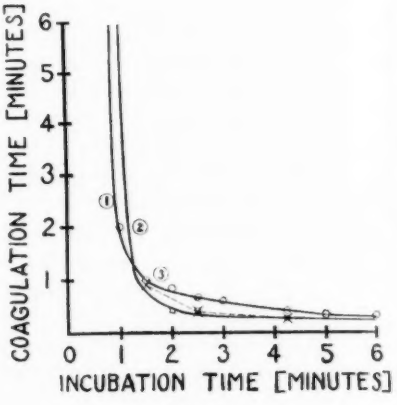

Fig. 2

Fig. 1. Comparison of the thromboplastic action of saliva with that of an extract of calf brain.

Curve *1*. Supernatant saliva after centrifugation. Curve *2*. Saline suspension of sediment from saliva used in curve *1* suspended in $\frac{1}{3}$ the original volume. Curve *3*. Fresh saline extract of calf brain.

Fig. 2. Comparison of the thromboplastic action of saliva with that of a suspension of leucocytes and blood platelets.

Curve *1*. Filtered saliva which was boiled one minute over an open flame. Curve *2*. Saline suspension of sediment obtained by centrifuging saliva and washing once. Curve *3* (dotted line). Saline suspension of washed leucocytes and platelets from beef blood.

Curve 1 shows the effect of the supernatant fluid from the same saliva. This is also true if a comparison is made between saliva and a suspension of washed leucocytes and platelets. Such a comparison is shown in figure 2, curves *2* and *3*.

The active material of saliva is probably a tissue extract. This is indicated by the fact that the sediment from centrifuged saliva is highly active in thromboplastic properties (see fig. 1, curve *2*, and fig. 3, curve *1*).

From figure 3 (curves *1* and *2*) it may be seen that the centrifuged sediment is about thrice as active as is whole saliva. The sediment consists of cellular material and this is probably the source of the thromboplastin in the fluid saliva.

Saliva may be dried in a vacuum oven with little loss of activity. The results of such an experiment are plotted in curve *4* of figure 3. It is stable in the desiccated state, whereas its activity is lost in a few days when it is liquid. The inactivation may be due to bacterial action since the addition of toluene serves to keep it longer.

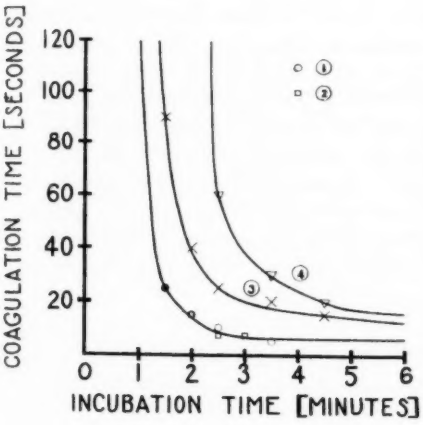

Fig. 3

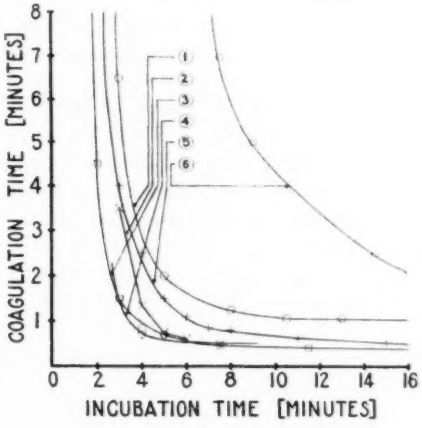

Fig. 4

Fig. 3. Experiments on the activity and stability of the thromboplastin of saliva. Curve *1*. Saline suspension of sediment obtained by centrifuging saliva. Curve *2*. Saliva concentrated 3-fold by ultrafiltration. Curve *3*. Whole, untreated saliva. Curve *4*. Saliva dried in vacuo at room temperature and then redissolved in distilled water.

Fig. 4. The influence of heating on the thromboplastic activity and stability of saliva.

Curve *1*. Supernatant saliva after centrifugation. Curve *2*. Same—heated 30 seconds at 100° and cooled in running water. Curve *3*. Same—heated 60 seconds. Curve *4*. Same—heated 3 minutes. Curve *5*. Same—heated 6 minutes. Curve *6*. Same—heated 10 minutes.

The influence of heating on the properties of saliva is shown by the curves given in figure 4. When centrifuged saliva is heated at 100° its activity is greatly enhanced during the first few minutes and then it is gradually lost. Very little activity is left after 10 minutes' heating (fig. 4, curve *6*), and none after 20 minutes. The maximum activity obtained by heating (fig. 4, curves *3* and *4*) is almost equal to that of the whole saliva (where the cellular elements are still present). The initial

increase of activity on heating may be attributed to the hot liquid extracting more active material from fragments of cells that remain suspended even after centrifuging. Undoubtedly the active material is steadily undergoing heat destruction from the start of the experiment.

The thermolabile nature of the active material together with its probable origin in the tissues leads one to suspect it may be a protein. This is supported by the observation that it is not dialysable. Saliva can be dialyzed for 24 hours in cellophane bags against running distilled water without loss of activity, also its activity can be increased by ultrafiltration (fig. 3, curve *2*).

Another experimental evidence, which favors the view that the active substance in the saliva is of protein nature, is that all of the activity is removed from solution by full saturation with ammonium sulfate, and that the active material may be recovered from the precipitate. The thromboplastin of saliva does not appear to be lipoidal. Ether or benzene extracts of saliva showed no thromboplastic activity. This perhaps may be due to the minute amounts extracted. However the residue from the extracts are not as active as the original material. Therefore the possibility exists that the active material contains a lipoid combined with a protein and that small amounts of the lipoid are being removed by extraction with the lipoid solvents.

SUMMARY

1. Saliva owes its blood coagulation-accelerating properties to the presence of a substance acting as a thromboplastin.

2. The active material in saliva is probably of cellular origin.

3. It appears to be protein in nature, possibly a lipo-protein.

4. It can be partially purified by ammonium sulfate precipitation and dialysis followed by desiccation at room temperature, in which condition it is fairly stable.

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THE EFFECTS OF FORMALIN ON THE THYROID STIMULATING AND GONADOTROPIC HORMONES OF CATTLE ANTERIOR PITUITARY GLANDS[1]

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Anterior pituitary gland preparations may exert on ovaries the following effects: 1, promotion of growth and full maturation of large follicles; 2, processes of luteinization of theca interna, of theca remnants, and perhaps also of ordinary connective tissue cells situated in the medulla of the ovary, and of granulosa. There may occur in addition a premature enlargement of the granulosa in not yet fully developed and even in small follicles; it is uncertain whether this change represents a maturation or a luteinization process; however, the second alternative is more probable, because this change never occurs in association with a full maturation of large follicles, but only in combination with luteinization processes. 3, atresin action (1).

Which of the various effects occurs depends on two sets of factors, namely, *a*, on the kind of anterior pituitary preparation used, and *b*, on the type of ovary used as test organ (1). The ovary of rabbit, rat and mouse very readily responds to anterior pituitary preparations with the growth and full maturation of follicles, while the guinea-pig ovary is an effective resonator to the agents which produce various luteinization processes and atresin effects and, in addition, growth-maturation effects, while the ovaries of rabbit, rat and mouse are supposed to respond to the action of lutein hormone with the formation of corpora lutea. The corpora lutea, however, can develop only after a preceding formation of fully mature follicles. Also, the thyroid gland of different species differs greatly in its ability to respond with structural changes to the action of the thyroid-stimulating hormone contained in various anterior pituitary preparations.

It would be very useful in the further analysis of the action of anterior pituitary hormones if we possessed preparations which exert exclusively, or almost exclusively, growth-maturation effects, and others which produce

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mainly luteinization or atresin effects, and if it were possible to separate the thyroid-stimulating action from the various changes induced in the ovaries. We have found that if we use the guinea-pig ovary as test organ it is readily possible to obtain anterior pituitary preparations which produce the follicular growth-maturation effect, either exclusively or with only a very slight admixture of the luteinizing process. By immersing a single cattle anterior pituitary gland either to the action of a formalin solution (2), or to the successive action of, first, urea and then glycerin (3), and implanting pieces of glands thus treated into guinea pigs weighing about 180 to 190 grams, on four or five consecutive days, pure growth-maturation effects can be obtained in a large proportion of the animals. Of these two methods the application of urea and glycerin seems to be the more effective one. While there are indications that also certain other solutions would act in a similar manner, the two methods mentioned are, so far, the only ones which have been studied more thoroughly by us. In the present investigations we have attempted to determine some of the variable factors which influence the effects exerted by cattle anterior pituitary glands previously treated with solutions of formalin.

Fresh hypophyses of cattle were cleaned by immersing them for a few minutes in 95 per cent alcohol and washing them with sterile 0.9 per cent NaCl solution. Then the capsule surrounding the gland was removed and the anterior and posterior lobes were separated with sterile instruments. The anterior lobes were divided into four approximately equal parts and these pieces were placed in a sterilized flask containing 250 cc. of a formalin solution of known concentrations and pH. The flasks were kept at 37°C. for three days in some experiments and for seven days in others. In order to avoid the possible effects of substances serving as buffer solutions on the hormones of the anterior pituitary lobes, the pH of the solution, after having been determined colorimetrically, was tested daily and brought back to the original state in case a deviation had taken place. At the end of the period of formalin action the four parts of the anterior pituitary lobe were removed from the solution and placed in a sterile Petri dish. The formalin solution was decanted and several cubic centimeters of a sterile 0.9 per cent NaCl solution were added, in order to wash off the remaining adhering formalin. One of the four gland pieces was then divided into four approximately equal parts and these pieces, each representing one-sixteenth of a whole gland, were implanted subcutaneously into young female guinea pigs with initial weights varying, as a rule, between 170 and 190 grams. On the following three days the remaining pieces, which in the meantime had been kept at about 4°C., were implanted in a similar manner. As a rule the examination of the organs took place on the fifth day, one day after the last implantation. Thyroids, uterus, cervix and vagina, adrenals and mammary glands were

imbedded in paraffin and cut into sections; the ovaries were cut into serial sections. If we exclude those experiments which for various reasons could not be used, sixty-seven guinea pigs served for these investigations, in which the following variable factors were considered: 1, the pH of the formalin solution; 2, concentration of formalin; 3, the number of days during which the gland pieces were exposed to the action of the formalin; 4, the seasons of the year in which the experiments were carried out, and 5, the initial weight and changes in weight of the guinea pig during the course of the experiment. The pH of the formalin solution was found to be the most important variable factor if the other factors did not exceed a certain range of variations.

Table 1 shows the effects of variations in the pH of the solutions and in the seasons at which the experiments were carried out. Summer experiments were done during June, July and August; there were also included two experiments made during the last days of April and in the beginning of May. Winter experiments were carried out during November, December and January. The results were considered positive when the ovaries showed fully mature follicles, when lutein effects were entirely or almost entirely lacking, and atresin effects were likewise absent. The thyroid gland was in a resting state, while under these conditions vagina, cervix and uterus, as a rule, showed the typical signs of heat. Experiments were considered negative when maturation of follicles was lacking but when there were large, or almost large, preserved follicles, and when any except rudimentary luteinization processes were absent. As a rule the thyroid gland showed no hypertrophy and uterus and vagina were inactive. Intermediate results were those in which fully mature follicles were not seen, but in which there were large preserved follicles, with perhaps a slight beginning maturation of large follicles; in such cases there was a little enlargement of vagina, cervix and uterus as an indication that a small amount of oestrogen in excess of the average amount had been given off by the ovarian follicles, due to the stimulating effect of the implanted anterior pituitary substance. However, in these intermediate cases this stimulation was not strong enough to cause complete heat changes in uterus and vagina.

We may conclude that the pH at which formalin acts is of importance in determining the results. The optimum is at pH 4 and pH 6; next in suitability is pH 8. At pH 2 and pH 10 no positive results were obtained. This is not due to the fact that pH 2 and pH 10, as such, destroy the follicular growth and maturation effect. Other experiments have shown that without exposure of the cattle anterior pituitaries to formalin solutions maturation effects may still be obtained in 0.9 per cent NaCl solutions at pH 2 and pH 12.

During the winter months better results were obtained on the whole,

than during the summer months. This difference was especially pronounced in the experiments with pH 4, where during the winter nine positive results and only one negative result were obtained. However, in these cases the animals used during the summer months evidently had suffered much from the heat, as is indicated by a greater than the average

TABLE 1

pH	NUMBER	RESULTS		
		Positive	Intermediate	Negative
Winter experiments				
2	7	0	0	7
4	11	9	1	1
6	6	4	1	1
8	6	3	2	1
10	5	0	0	5
Summer experiments				
2	7	0	4	3
4	7	0	3	4
6	6	3	1	2
8	7	1	3	3
10	5	0	2	3
Total number of experiments	67	20	17	30
Total winter experiments	35	16	4	15
Total summer experiments	32	4	13	15
Experiments at pH 4 and pH 6				
Winter	17	13	2	2
Summer	13	3	4	6
Total	30	16	6	8
Experiments at pH 4, pH 6 and pH 8				
Total	43	20	11	12

loss in weight. But also in the other groups where such a difference in weight changes did not exist between summer and winter guinea pigs, more positive results were obtained in the winter experiments; here the large majority of the winter experiments carried out at pH 4 and pH 6 therefore were positive.

Full maturation of follicles was obtained with concentrations of 0.25

per cent, as well as 0.5 per cent, 0.75 per cent and 1 per cent formalin. But negative results seemed to be slightly more frequent in those experiments in which a 1 per cent solution of formalin was used; still, it is doubtful whether slight variations of this kind are of significance. Likewise there was no marked difference between experiments in which the anterior pituitary glands were exposed to the formalin solution for three days or for seven days. We may conclude that a 0.25 per cent solution of formalin acting during a period of three days on an anterior pituitary gland in vitro is able to produce the typical changes, but that exposure up to seven days is not injurious to the follicular growth-maturation hormone.

The changes in weight which took place in the course of the experiments, as well as the weights of the animals at the beginning of the experiments, were not factors of special significance. There was no definite correlation between the changes in the ovary and the weights within the range of variations used in our experiments. However, as stated above, it is probable that a greater average loss in the summer experiments at pH 4 was partly responsible for the less favorable results obtained in this series. There is likewise some indication that also in other experiments the results were not quite so good in guinea pigs which did not gain definitely in weight during the experiments, as in animals which did gain in weight and in which also the initial weight was greater. But on the whole, the effects of weight were not decisive as far as the condition of the ovaries is concerned.

As to changes in the ovaries other than maturation of follicles, the following effects were observed under the influence of implanted anterior pituitary glands treated previously with formalin. At pH 2, lutein effects were abolished and the thyroid-stimulating hormone was destroyed; but in two summer experiments there was perhaps a trace of thyroid hypertrophy. At pH 4, very slight luteinization changes were found in a few cases; in only one animal was there possibly a trace of thyroid hypertrophy. At pH 6 very slight luteinization effects were noted in two animals, in which also fully mature follicles were found. In one of these cases there was a slight enlargement of the theca interna in completely atretic follicles and in the other case a very slight enlargement of the interstitial gland cells in the medulla of the ovary had taken place. The thyroid-stimulating hormone was destroyed in all these instances. At pH 8 the effects of the thyroid-stimulating hormone had been destroyed in all animals. In three of these guinea pigs there were present very slight degrees of luteinization of the theca interna of atretic follicles or of the interstitial gland cells, and in one of them fully mature follicles were obtained. At pH 10 the thyroid-stimulating hormone was inactive and only in one case was there a very slight enlargement of the interstitial gland cells noticeable.

In none of the 67 guinea pigs were typical atresin effects observed; the

formalin treatment abolished these together with almost all the other hormone effects, except the growth and maturation of follicles. However, in a number of cases which were negative as far as the development of entirely mature follicles was concerned, the follicles did not reach their full development, but, instead, granulosa degeneration set in at a slightly earlier stage than normal. Such results may have been due to unfavorable conditions of nutrition, which, as we have stated previously, affect very readily the growth of follicles (4).

SUMMARY AND CONCLUSIONS

Our experiments have shown that by immersing single cattle anterior pituitary glands in formalin solutions for three or seven days and then implanting them in fractions into guinea pigs, it is possible to suppress entirely, or almost entirely, the atresin and luteinization effects which are exerted normally by such glands in the ovary of the guinea pig, and to produce instead full maturation of follicles, a result which is not obtained if we implant the untreated anterior pituitary gland. Likewise, the effects of the thyroid-stimulating hormone are abolished in almost all cases by formalin action. While the untreated cattle anterior pituitary gland produces only a very slight or no enlargement of the uterus, and no proliferation or only a very incomplete one in vagina, cervix and mammary gland, after implantation of the formalin-treated gland full heat changes occur in these organs. As we shall later show, it is possible to preserve the anterior pituitary glands thus modified by formalin action for long periods of time, so that they can be used for the induction of heat changes in the guinea pig.

We have investigated certain variable factors which influence the effectiveness of the formalin treatment. The pH of the formalin solution which acts on the gland tissue in vitro was found in this respect to be of great significance; also the season during which the experiment is carried out seems to be of some importance. The other factors which we tested exerted no or very little effect within the range supplied in our investigations.

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NERVE IMPULSE FREQUENCY AND ITS RELATION TO VASOMOTOR REFLEXES[1]

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Electrical stimulation of peripheral nerves may elicit either an increase or a decrease in the systemic blood pressure or there may be no change at all. The conditions which determine whether a stimulus is to be followed by a pressor or depressor vasomotor response have been the subject of much investigation and some controversy.

In most previous investigations of the effect of electrical stimulation of peripheral nerves upon vasomotor responses, some modification of the inductorium has been employed as a stimulator, and, consequently, there has been little or no control of the factors which determine the characteristics of the stimulation. For this reason, the results obtained with such techniques are open to some question.

In the present investigation, the problem of vasomotor responses elicited by electrical stimulation of peripheral nerves has been studied with the use of a thyratron stimulator which permits independent control and variation of all the characteristics of stimulation.

METHOD. There are four factors which determine the characteristics of repetitive electrical stimulation. They are: 1, the peak amplitude of each discharge, which may be designated as the *intensity*; 2, the duration and voltage configuration of each discharge, which may be designated as the *configuration* (the *pulse-frequency* of Dusser de Barenne and McCulloch (1937)); 3, the frequency of repetition of discharges, which may be designated as the *frequency* (the *pattern-frequency* of Dusser de Barenne and McCulloch (1937)), and 4, the total period during which the repetitive stimulation is applied, which may be designated as the *duration*.

To study the effect of each of these characteristics of stimulation, a device must be employed which permits independent control and variation of all the characteristics. This requirement is satisfied by the thyratron stimulator which has been used in the present investigation. Figure 1 is a diagram of this stimulator, which is a modification of that described by Schmitt and Schmitt (1932).

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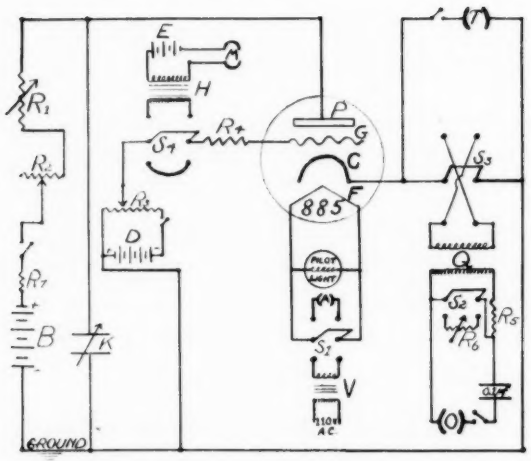

Fig. 1

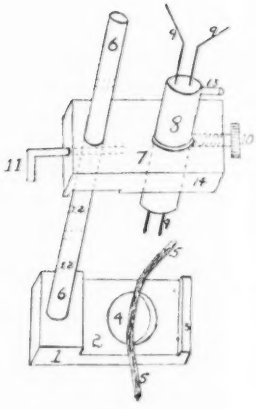

Fig. 2

Fig. 1. Wiring diagram of thyratron stimulator. The type RCA 885 tube was employed. *A*, jack for connecting 2.5 volt storage battery; *B*, dry battery of suitable voltage depending upon maximum intensity and frequency of stimulus desired; *C*, cathode of 885 tube; *D*, 22½ volt radio "C" battery for grid bias; *E*, dry battery for positive voltage on grid when using outside mechanical control of stimulator; *F*, filament of 885 tube; *G*, grid of 885 tube; *H*, step-up transformer; *K*, decade condenser of range 0.001 to 1.0 microfarad; *M*, jack for outside mechanical control of stimulator; *O*, jack for connecting output leads; *P*, plate of 885 tube; *Q*, air core transformer (Harvard inductorium with secondary entirely covering primary); *R1*, variable resistance of 5 megohms in steps of 100,000 ohms; *R2*, 150,000 ohms potentiometer; *R3*, potentiometer for varying grid bias; *R4*, 200,000 ohms grid resistor; *R5*, 1000 ohms fixed resistor; *R6*, 1–10,000 ohms L & N decade resistance; *R7*, 10,000 ohms resistor; *S1*, switch for changing filament supply from transformer to 2.5 volt storage battery; *S2*, switch for connecting shunt resistance *R6* across output terminals; *S3*, switch for reversing polarity of stimulation; *S4*, switch for introducing outside mechanical control of stimulator; *T*, jack for connecting signal magnet or time-recording device; *V*, 2.5 volt filament transformer.

Fig. 2. Shielded non-polarizable Ag-AgCl electrodes. *1*, base; *2*, recess in which the nerve *5* rests; *3*, raised ledge which prevents the crushing of the nerve by the slide *7* when it descends into stimulating position; *4*, well into which electrodes *9* descend; *5*, nerve; *6*, upright post on which slides *7* into which is inserted the electrode holder *8* which contains electrodes *9* which are Ag-AgCl; *10*, set screw to hold *8* in any position so that electrodes *9* may make good contact with nerve *5*; *11*, pin for holding *7* in any horizontal level; *12*, slots in post *6* to fit pin *11*; *13*, lever to rotate *8* to desired position; *14*, raised portion of *7* which rests on ledge *3*.

Before use, the electrode holder *8* is removed and immersed in hot hydrochloric acid. The electrodes, *9*, thus acquire a coating of silver chloride very rapidly. It is sometimes necessary to deposit fresh silver electrolytically from a bath of silver nitrate before immersion in the hydrochloric acid. The electrode holder is reinserted into the slide *7* which is maintained in a raised position by the pin *11*. The well *4* is inserted under the nerve and the entire apparatus is held in position by means of a clamp attached to a flexible lead rod. The slide *7* is lowered so that the raised portion *14* rests on the ledge *3* and the holder *8* is rotated by the lever *13* until the electrodes *9* make contact with the nerve *5*. The set screw *10* is then tightened and the pin *11* is inserted.

The entire apparatus is made of bakelite. The electrode holder *8* is made of linen bakelite which does not deteriorate on immersion in hot hydrochloric acid.

During the present series of experiments, the configuration was kept constant, while the other factors—intensity, frequency and duration—were varied separately. That these factors could be varied independently was demonstrated by frequent analyses of the output of the stimulator with a cathode ray oscillograph.

Experiments were performed on forty-one cats. In the first group of twenty-four cats, the sciatic nerve and some of its branches were stimulated with the electrode illustrated in figure 2, designed to permit stimulation of uncut nerves, as well as with the conventional type of shielded silver electrode. Blood pressure was recorded by a mercury manometer connected to a cannula in the left carotid artery, and respiration, by a tambour connected with a tracheal cannula. Thirteen of the animals were anesthetized with ether during the stimulation, eleven were decerebrated, and two of the etherized and two of the decerebrate cats were curarized. During curarization, the respiration was artificially maintained.

In a second group of eight cats, a spinal cord transection at T10 or T11 was performed one to three days before the experiment and the cats were allowed to recover. In these animals, a modified Sherrington electrode, with a housing made of Lucite, was attached to the central end of the left ulnar nerve under ether anesthesia and a cannula was inserted into the left femoral artery for recording blood pressure. The animal was allowed to recover from anesthesia and the ulnar nerve was then stimulated. In a few cases, respiration was recorded by a tambour connected with a pneumograph placed around the thorax.

In a third group of nine animals, action potential observations on the sciatic nerve were made with a cathode ray oscillograph.

Results. *A. Vasomotor responses* (fig. 3). *1. Effect of varying the frequency of stimulation.* In the experiments on decerebrate, anesthetized and curarized cats, it was found that a depressor vasomotor response could usually be elicited by electrical stimulation of the central end of the sciatic and tibial nerves or the uncut nerves with intensities ranging from the weakest effective stimulation (0.5–2.0 volts) to an intensity of 100 volts or more, if appropriate low frequencies, usually below 30 per second, were employed. With appropriate high frequencies, usually above 70 per second, a pressor response could be elicited over the same wide range of intensities. At any constant intensity, the direction of the vasomotor response could usually be reversed by changing from an appropriate low frequency to an appropriate high frequency and vice versa.

In some cases, a depressor response was preceded by a slight initial rise in blood pressure. A pressor response was never preceded by a fall in blood pressure, but was sometimes followed by a fall after cessation of the stimulation. When an appropriate pressor stimulus was applied, the

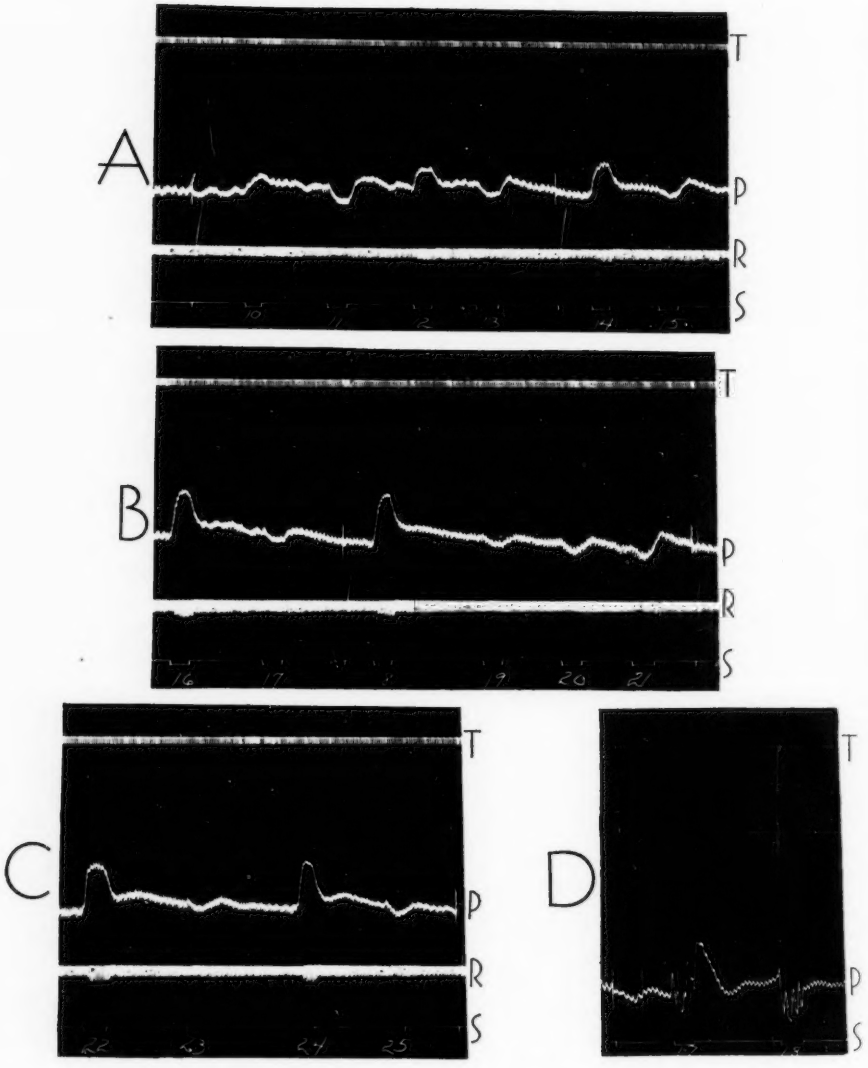

Fig. 3. Kymograms of blood pressure and respiration in the cat at various intensities and frequencies of stimulation of the sciatic and ulnar nerves. *A*, *B* and *C* are continuous records of stimulation of the right sciatic nerve in an etherized male cat with the thyratron stimulator. The motor threshold in this cat was about 500 millivolts. The intensities of the stimuli are given in volts and the frequencies in shocks per second. *T*, time in seconds; *P*, blood pressure; *R*, respiration; *S*, duration of stimulation.

(10) 1.5 volt, 250/sec.; (11) 1.5 volt, 23/sec.; (12) 2.5 volts, 250/sec.; (13) 2.5 volts,

blood pressure usually rose promptly and remained at a high level throughout the entire duration of the stimulation, beginning the return to normal only when the stimulation was discontinued. With an appropriate depressor stimulus, the blood pressure fell and remained at the low level until the stimulation ceased, whereupon it returned to its original level.

For any particular intensity of stimulation, the magnitude of the pressor response, elicited by appropriate high frequency of stimulation, was usually greater than the magnitude of the corresponding depressor response elicited with the same intensity of stimulation but lower frequency. After a depressor response was obtained with appropriate low frequency stimulation, further decrease in the frequency at the same intensity usually resulted in an increase in the magnitude of the depressor response. However, the direction of the response to appropriate frequency of stimulation was very consistent and usually unmistakable.

The effects of stimulation, as described above, are much modified by changes in the condition of the animal with respect to the type and depth of anesthesia employed, the body temperature, the room temperature in animals with temperature regulating mechanism destroyed, and drug administration. Incidental observations concerning such modifications of response were made during the present experiments as follows: a, *Ether anesthesia:* In deeply anesthetized animals with a gradually falling level of blood pressure, the only response which could be elicited by any intensity or frequency of stimulation was a depressor response and, in many cases, no response at all was elicitable. The removal of the anesthetic and the recovery of the animal were followed by a condition in which, once again, both pressor and depressor responses could be elicited by appropriate frequencies of stimulation. b. *Curare:* In the early stages of curarization, both pressor and depressor responses could be obtained, but, in the later stages, when the level of blood pressure became low, only pressor responses could be elicited from the sciatic nerve. c. *Body temperature:* In decerebrate animals, in which the rectal temperature rose to 38°–40°C., it was observed that, in some cases, only pressor responses could be elicited by stimulation of the sciatic nerve, while in other such cases of high rectal temperature, only depressor responses could be elicited. In one case, in which a high rectal temperature (40°C.) was found to be due to a hot operating lamp, removal of this source of heat resulted in a fall of rectal

23/sec.; (14) 12 volts, 250/sec.; (15) 12 volts, 23/sec.; (16) 20 volts, 250/sec.; (17) 20 volts, 23/sec.; (18) 46 volts, 250/sec.; (19) 46 volts, 23/sec.; (20) 46 volts, 10/sec.; (21) 46 volts, 5/sec.; (22) 56 volts, 250/sec.; (23) 56 volts, 23/sec.; (24) 56 volts, 250/sec.; (25) 56 volts, 23/sec.

D, record of stimulation of the left ulnar nerve in a spinal unanesthetized cat. Same symbols as in *A*, *B*, *C*.

(17) 19 volts, 154/sec.; (18) 19 volts, 14/sec. In both (17) and (18) there was vocalization and movement of the head.

temperature. In this animal, when the rectal temperature was above 38°C., only pressor responses could be elicited. When the temperature fell below 38°C., in the range 37°–38°C., both pressor and depressor responses could be elicited with appropriate frequency of stimulation. When the temperature fell below 37°C., only depressor responses could be elicited. Throughout these observations on warming and cooling, the general blood pressure level remained at about 120 mm. Hg.

In the spinal, unanesthetized cat, the factors which fortuitously changed the level of the blood pressure, independently of any stimulation of the ulnar nerve, had to be taken into account in interpreting the effects of stimulation. Fortuitous movements or struggling of the animal resulted in a rise in the blood pressure level. Vocalization or attempts at vocalization were followed by marked decreases in blood pressure. Each vocalization was accompanied by a sharp drop in blood pressure of 30–50 mm. Hg followed by an immediate return to normal.

The effects of varying the frequency of stimulation of the left ulnar nerve in the spinal, unanesthetized cats were essentially similar to the effects of stimulating the sciatic nerve in decerebrate, anesthetized and curarized animals, as described above. However, in addition to vasomotor responses, the unanesthetized cats presented the opportunity of observing responses usually regarded as indicators of pain such as struggling and vocalization, sometimes accompanied by clawing and biting. The word "pain" as used in the following statements refers to such visible manifestations.

In these unanesthetized spinal cats, if the intensity of stimulation were between 2.5 and 4.0 volts, frequencies of about 15 per second elicited depressor responses while frequencies of about 150 per second elicited pressor responses, with any constant intensity in the range mentioned. These vasomotor responses were unaccompanied by "pain." At intensities between 4 volts and 50 volts, high frequencies elicited marked pressor responses accompanied by "pain" and low frequencies, at the same intensities, elicited no "pain" and no change in blood pressure or an occasional fall. At intensities above 50 volts, low frequencies usually, but not invariably, elicited some "pain" accompanied by an increase in blood pressure, but this effect was never so marked as with high frequencies at the same intensity, and it did not occur at all if the animal was made to lie quietly by stroking its head during the stimulation.

It is an interesting fact that the sharp drops in blood pressure of 30–50 mm. Hg accompanying vocalization have little or no effect upon the direction of the response during stimulation. Thus, in figure 3 (D), vocalization occurs during the high frequency as well as during the low frequency stimulation and the sharp dips in blood pressure are quite evident, yet in one case there is a sharp rise in the blood pressure level and in the other case a sharp fall.

2. Effect of varying the intensity of stimulation. In order to relate the stimulation intensities employed in the present investigation to the threshold for the most irritable fibers in the sciatic nerve, determinations of this threshold were made by two methods: 1. Action potential observations on the sciatic nerves of decerebrate cats indicated that the threshold for the most irritable fibers in the nerve varied between less than 40 millivolts and 200 millivolts. 2. The threshold of the motor fibers of the sciatic nerve, presumably the most irritable fibers, was determined by noting the lowest intensity of stimulation which would evoke a twitch of the hind leg muscles which was just perceptible to the naked eye or to the touch of a finger placed in contact with the muscle. The motor thresholds ranged from 200 millivolts to 500 millivolts. The discrepancy between the results of these two methods is undoubtedly due to the relatively crude technique used for determining the motor thresholds.

In the decerebrate, anesthetized and curarized animals, in most cases, a vasomotor response could be obtained with a stimulus intensity of 1 to 2 volts. However, in a few cases, a weaker stimulus, 500 to 750 millivolts, was effective. In animals with rectal temperature of 37°-38°C., which were not too deeply anesthetized and not cyanotic, the effect of increasing the intensity of the stimulus was to increase the magnitude of pressor responses and to decrease the magnitude of depressor responses up to a limiting value, beyond which further increases in intensity failed to elicit further changes in magnitude. However, it was not possible to convert a depressor response into a pressor one by merely increasing the intensity of stimulation, provided that the frequency of stimulation was appropriate for a depressor response, as discussed above.

In animals in which, because of any of the conditions of overanesthesia, hyperpyrexia, hypoprexia or curarization, as described above, only depressor responses could be obtained, the magnitude of the depressor response usually increased with increasing intensity of stimulation, and stimuli of frequency appropriate for pressor responses usually had no effect. Conversely, when only pressor responses were obtainable, the magnitude of the response usually increased with increasing intensity of stimulation, and stimuli of frequency appropriate for depressor responses usually had no detectable effect, except in a few rare instances, where a slight pressor response was obtained. In these few instances, after further decrease of the frequency, usually no pressor effects could be elicited.

The effect of varying the intensity of stimulation in the experiments on the unanesthetized animals has been described above in connection with the description of variations in frequency.

B. Respiratory responses (fig. 3). *1. Effect of varying the frequency of stimulation.* High frequency (pressor) stimulation was usually accompanied by increased rate and amplitude of respiration during the period

of stimulation. There was also struggling during the period of high frequency stimulation in lightly anesthetized animals, but this struggling terminated upon cessation of the stimulation. Low frequency (depressor) stimulation usually resulted in a diminution of the frequency and amplitude of respiration, sometimes to the point of apnea, during the period of stimulation. Upon cessation of the low frequency stimulation, rapid respiration of high amplitude usually supervened and this was usually accompanied by struggling in lightly anesthetized animals, although there had been no struggling during the period of stimulation.

2. Effect of varying the intensity of stimulation. It was observed that the threshold for respiratory effect was usually different from that for vasomotor effect, the respiratory threshold being usually the higher one, although, in a few instances, it was the lower. Increased intensity of high frequency stimulation usually augmented the amplitude of the respiratory response, but increased intensity of low frequency stimulation usually had no effect on the magnitude of the respiratory response. The possible relation between respiratory responses and vasomotor effects will be discussed below.

Discussion. Certain previous observers (Kronecker and Nicolaides, 1883; Gruber, 1917; Hunt, 1918; Ogata and Vincent, 1919; Vincent and Thompson, 1928) have indicated that the frequency of stimulation may play an important rôle in determining the direction of vasomotor response to electrical stimulation of peripheral nerves, while others (Hunt, 1895; Martin and Lacey, 1914; Vincent and Thompson, 1928; O'Leary, Heinbecker and Bishop, 1935) have thought that the intensity of stimulation is a determining factor.

The present results support the view that the sign or direction of the vasomotor response may be determined by the frequency of stimulation at a constant intensity. In the present investigation, the effective rising phase of each single discharge lasted for about one-fiftieth of a millisecond, and the entire discharge period was very brief. Since the period of each stimulus was so short, it may be permissible to assume that each stimulus corresponded to a single impulse in the fibers which it stimulated and that the stimulation frequency was the same as the nerve impulse frequency and, consequently, that the sign of the vasomotor response depends upon the nerve impulse frequency.

It should be noted that the belief in the importance of the intensity factor in stimulation is a necessary corollary of the belief that there are specific peripheral fibers whose centripetal impulses elicit specific vasomotor responses. Those who maintain that specific peripheral fibers subserve specific vasomotor effects generally assign the rôle of the pressor fibers to the smaller, less irritable, more slowly conducting fibers. The factor which determines the capacity of a nerve fiber to conduct impulses

at any frequency is the rate of recovery of the fiber from the effects of a stimulus, i.e., the refractory period (Erlanger and Gasser, 1937, p. 173). Although the available data on the relation of fiber size and conduction rate to duration of refractory period is variable, the indications are (Erlanger and Gasser, 1937, pp. 47–50) that, the smaller the fiber and the slower its conduction rate, the longer is the duration of its refractory period, and consequently, the lower is the maximum frequency at which it can conduct impulses. Therefore, even if it were true that, at the higher frequencies in the present experiments, some of the fibers were unable to respond so rapidly, it would be the smaller "pressor" fibers which would be the first to fail to respond, and the effect of increasing the frequency should be a decrease in pressor response rather than an increase as was actually observed.

In order to demonstrate that the groups of fibers in a mixed afferent nerve, activated by a constant intensity of stimulation, do not change in kind, number or activity when the frequency of stimulation is varied between the limits required to elicit reversal of vasomotor response, it would be desirable to study the action potentials of the various groups of fibers under the actual conditions of stimulation at the various frequencies. Such a study would be especially important with regard to the C fibers, and has been attempted but has not been completed because of certain inherent technical difficulties. To mention the most serious of these difficulties, in studying the C potentials in a mixed nerve, it is necessary to use a conduction distance between the stimulating and lead electrodes such that the A and B potentials do not confuse the C potential picture. At such a conduction distance, the temporal dispersion of the C potentials is so great that a frequency of stimulation higher than 20 or 30 per second cannot be employed without distortion of the C potential by the stimulation artefacts and by overlapping of the C potentials.

However, although actual data concerning the action potential changes in mixed nerves under the conditions of the present experiments is not available, there is no reason to believe from previous work in this field that frequencies up to 100 per second produce any change in the number or kinds of fibers activated at constant intensities of stimulation. Therefore, since reversal of vasomotor response could be elicited by appropriate frequencies below 100 per second at constant intensity of stimulation, there is no reason to believe that the reversal of response is not occasioned by changes in the nerve impulse frequencies rather than by changes in the kind of nerve fibers effectively conducting centripetal impulses. It would seem that the nerve impulse frequency rather than the nerve fiber type determines the sign of the vasomotor response under such conditions of constant intensity of stimulation.

Since the number of fibers effectively stimulated to conduct centripetal

impulses depends upon the intensity of stimulation and since the magnitude of the vasomotor response also seems to be related to the intensity of stimulation, it would seem that the magnitude of the response is related to the number of fibers actively conducting centripetal impulses and is, therefore, dependent upon spatial summation. However, at any constant intensity of stimulation, the sign of the vasomotor response seems to be determined by the frequency with which impulses reach the central nervous system, i.e., by temporal summation.

There has been some question in the literature concerning the importance of the respiration in producing vasomotor effects. The striking effect of vocalization upon the blood pressure in an unanesthetized animal demonstrates the profound influence which respiratory or other mechanical factors may have upon the blood pressure. Yet it is very interesting to note, as illustrated in figure 3 (D), that the vocalization effect was present in similar degree in both the pressor and depressor responses but this did not alter the direction of the response as determined by the frequency of stimulation. The fact that the response reversals described above were obtained as well in curarized animals with artificial respiration as in normal animals under ether anesthesia provides additional support for the belief that the respiratory reflex phenomena may be distinct from the vasomotor phenomena and may not have any causal relation to the vasomotor responses. This is in accord with the results of Bishop, Heinbecker and O'Leary (1934).

Thus, although respiratory or other mechanical factors must be taken into consideration in interpreting the effects of stimulation, it is believed that the reversals of vasomotor response in the present investigation were determined by the characteristics of the stimulation applied to the afferent nerves rather than by the respiration.

SUMMARY

1. By the use of controlled stimulation with a thyratron stimulator, it has been demonstrated in cats under ether anesthesia, in decerebrate, in curarized, and in unanesthetized spinal cats that the sign or direction of the vasomotor response (pressor or depressor) elicited by electrical stimulation of the sciatic, tibial and ulnar nerves at constant intensity of stimulation is determined by the frequency of stimulation.

2. The magnitude of the vasomotor response has been shown to be related to the intensity of the stimulus, up to a limiting value.

3. The results indicate that respiratory reflexes may have a mechanism distinct from that of vasomotor reflexes and are not entirely responsible for the reversals of vasomotor response obtained with different frequencies of stimulation.

4. It has been demonstrated that, under appropriate conditions, elec-

trical stimulation of the ulnar nerve in the unanesthetized spinal animal may elicit vasomotor responses unaccompanied by struggling, vocalization, or other phenomena usually considered manifestations of pain in animal experimentation.

5. The relation of the present results to the theory of fiber specificity in vasomotor reactions is discussed.

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DIFFERENTIAL PRESSURES IN THE LESSER CIRCULATION OF THE UNANESTHETIZED DOG

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The prime function of vasoconstriction is to supply blood to active organs by depriving inactive organs of their usual quota. This alternative supply of blood to the different organs, depending upon their activity, is essential in the regulation of the greater circulation but it is difficult to see how it may be of use in the lesser circuit. All parts of the lung are equal and no advantage can be normally served by shunting blood out of one lobe and into another. A collapsed lung, however, is said to have a reduced supply of blood passing through it because the saturation of the mixed arterial blood is not diminished in proportion to the amount of inactive pulmonary tissue (1, 2). This, however, does not mean that vasoconstriction is the means used to stop blood flow to the collapsed area, for where one lung is collapsed in atmospheric pressure and the other expanded in a normal intrathoracic pressure, the pressure within the pulmonary tissues must be greater in the collapsed lung. This pressure in the pulmonary tissue would appear in the hemodynamic picture as peripheral resistance and would probably be adequate to shunt blood flow from the collapsed to the expanded lung. Indeed it has been shown (3) (also see below) that large increases (2 to 3 fold) in flow through the pulmonary bed can occur with very small changes in pressure. The accumulation of fluid in the alveoli probably raises the pressure within the pulmonary tissues sufficiently to block blood flow away from the diseased lung in pneumonia and thus to account for absence of arterial unsaturation commensurate with the degree of pulmonary consolidation (2).

The evidence brought out by Johnson et al. (3) seems to minimize the rôle of vasoconstriction in the hemodynamics of the lesser circulation. In view of its importance it was thought of interest to re-examine the question by means of differential manometers and to repeat the observations on animals that were completely unanesthetized while the curves were being recorded.

APPARATUS. The manometers used were of the "hypodermic" type (4, 5). In order to eliminate from the record the fluctuations of intra-

thoracic pressure the differential manometer described elsewhere (6, 7) was used. In this device the intrathoracic pressure was led from a small balloon in the thorax to an air chamber enclosing the front part of an ordinary "hypodermic manometer." The pressure in the pulmonary artery or vein was transmitted in the usual way to the manometer itself. The manometer registers the difference between the intrathoracic pressure (minus) and the blood pressure (plus). It responds only to those pressures which distend the blood vessel as it lies within the thorax and does not respond to the intrathoracic pressure changes themselves which, of course, are led simultaneously to both sides of the sensitive plate in the manometer.

The hemodynamics of the lesser circulation can be analyzed further by recording directly the difference in pressure between the arteries and the veins. To do this another type of differential manometer was devised (see fig. 1) (6). The venous pressure was led by a column of citrate through a leaden tube to the chamber at the right containing the mirror. The arterial pressure came in from the left and worked upon the opposite side of the membrane supporting the mirror. The manometer therefore cancels all simultaneous changes in pressure, such as those due to respiration, and records only the effective pressure gradient which produces flow through the pulmonary bed. Having the mirror immersed in fluid introduces an asymmetrical refractive medium into the optical system. The layer of water acts as a prism and the front curvature of the usual plano-convex mirror (4, 5) was robbed of its refractive power. These optical defects were overcome by mounting an oculist's prism and lens, as indicated, in the path of the beam so as to bring it to focus upon the camera.

METHODS. The first experiments were performed on dogs operated in the same way as those described by Johnson et al. (3). None of these experiments gave evidence proving that pulmonary vasoconstriction played a rôle in the hemodynamics of the lesser circulation. Crucial experiments were therefore repeated on animals prepared with angiostomy cannulae so that pulmonary artery and vein could be entered while the animal was unanesthetized.

The operation[1] differs from that described by London (8) and Daly (9). Entrance to the chest was made through the left 4th interspace. Artificial respiration and ether were given by intubation. A no. 26 French catheter was prepared as in figure 2 (bottom). An extra hole was cut in the wall of the catheter so as to open into a condom balloon tied near the end of the catheter as shown in the diagram. The catheter was stiffened temporarily by means of a 3 mm. brass wire curved at the end and was inserted through the glottis into the trachea. It was attached to the

[1] This operation was described before the American Physiological Society in March 1938. See this Journal **123:** 220, 1938.

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artificial respiration apparatus and the balloon inflated with each blast of etherized air, occluding the trachea. The balloon deflated after the blast, allowing the lungs to empty past the catheter. We found this method decidedly advantageous because the occlusion of the trachea occurs only during inspiration and the dead air space is greatly reduced. This results in 1, better ciliary drainage of the bronchial tree; 2, adequate alveolar ventilation with a minimum of lung movement and hence a minimum of interference with the operation, and 3, a minimum of cooling and drying of the animal by the reduced quantity of ether laden air.

The angiostomy cannulae which we used were in two parts. The perforated flange shown in two views to the left in figure 2 was sewed to the artery or vein at the points x in figure 3. The pericardium was slit and the perforations sewn to the vessel under the pericardium. The pericardium was brought up around the stem to share in the stresses. In most cases the chest was closed and the flanged bases allowed to heal in place firmly before the next steps were taken. A few successful operations were performed in one stage.

When time had elapsed for healing the chest was again opened and the

Fig. 1. *Differential manometer* consists of a water filled chamber, shaped so as to be easily freed of bubbles, and fronted with an optical glass window. Into this is screwed a hypodermic manometer. Pressure is transmitted to the manometer from the pulmonary artery, to the chamber from the vein. The pressure recorded by the mirror is the differential between them. At the extreme right are an oculist's lens and prism used to bring the light reflected from the mirror to a focus on the camera.

Fig. 2. (Above) Angiostomy cannula. To the left two views of the flange which was sewed to the vessel and allowed to heal in place. To the right is the cannula proper which was screwed into the flange at a later operation and sewed into place as seen in figure 4.

Fig. 2. (Below) The tracheal catheter for artificial respiration. The balloon occludes the trachea during inspiration, opens it during expiration.

Fig. 3. Diagram of heart and vessels to show position of flanges, x. Vertical line indicates position of left phrenic nerve.

Fig. 4. Diagrammatic section showing relation of cannulae.

Fig. 5. Upper curve, carotid pressure. Second curve, pulmonary arterial pressure. Third curve, pulmonary arterial pressure minus intrathoracic pressure. During the first nine respiratory cycles the tracheal cannula was obstructed, after which the respiration returned to normal. Time, 10 seconds.

Fig. 6. Upper curve, carotid pressure. Second curve, pulmonary venous pressure. Lower curve, pulmonary venous pressure minus intrathoracic pressure. Occlusion of tracheal cannula as in figure 5. Time, 10 seconds.

Fig. 7. Upper curve, carotid pressure. Lower curve, carotid pressure minus intrathoracic pressure.

Fig. 8. Upper curve, carotid pressure. Middle curve, pulmonary arterial pressure. Lower curve, pulmonary arterial pressure minus intrathoracic pressure. In the middle of the curve the lungs are suddenly inflated with air under positive pressure. Time, 10 seconds.

cannulae bases exposed. Separate stab wounds were made at some distance from the main incision. Care was taken to stretch the skin to one side before making the stab sounds, so that in sewing up the skin the stitches would not be directly above the end of the cannulae. A cannula, shown at the right in figure 2, was selected long enough to reach from the base to the stab wound so as neither to compress the vessel nor to exert undue tension upon it (see fig. 4). The cannula was screwed into the base, the large head buried in the subcutaneous tissue and stitched in place to prevent rotation. Next, three inch needles were carefully inserted through the cannulae and blood drawn from the vessels. When it was clear that the vessels were fairly entered, measurements were made of the depth of needle insertion for future reference. The skin was then sewed over the cannula head, the chest closed, and the animal allowed to recover.

Results. *Experiments in which either pulmonary arterial or pulmonary venous pressures were balanced against the intrathoracic pressures (anesthetized dogs).* Figure 5 shows from above downwards simultaneous records of *a*, the carotid pressure; *b*, the pulmonary arterial pressure, and *c*, the pulmonary arterial pressure minus intrathoracic pressure. The upper two records were taken by ordinary "hypodermic manometers." For the lower record the pressure was transmitted from the same pulmonary cannula to the rear chamber of a differential manometer, and the intrathoracic pressure was transmitted by an air column to the front chamber of the manometer.

During the first nine respiratory cycles the tracheal cannula was obstructed and the respiratory intrathoracic pressure changes were abnormally large. During the last part of the record the respiration returned to normal. The pulmonary arterial pressure shows a sharp inspiratory decrease which becomes less when the breathing is not labored. The differential pressure, on the other hand, is increased with each inspiration. The differential pressure is in this case a measure of the force which distends the pulmonary artery whereas the pulmonary arterial pressure is this same force with the changes of intrathoracic pressure superimposed.

The increase in net systolic, diastolic and pulse pressure at least during forced inspiration, indicates that the right ventricle must be better filled and must be putting out a larger stroke volume. Blood must therefore be aspirated into the thorax during forced inspiration and be put out into the lungs immediately.

Figure 6 shows the carotid pressure above, the pulmonary venous pressure in the middle, and the pulmonary venous pressure minus the intrathoracic pressure below. At first the tracheal cannula was obstructed, later freed, and the respiration returned to normal. The respiratory

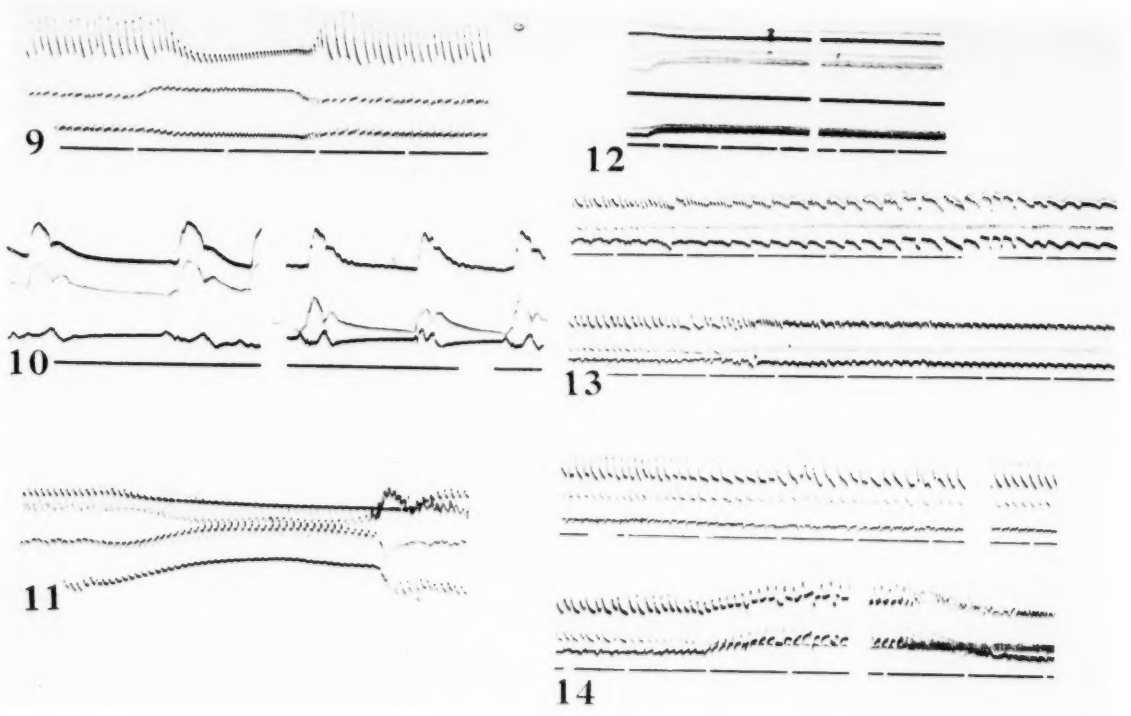
fluctuations in the venous pressure do not appear clearly in the differential pressure. There are only very small respiratory changes in the pressure which distends the pulmonary veins.

The respiratory changes in the systemic pressure are roughly in phase with the fluctuations of intrathoracic pressure. In some of our records (figs. 5, 6) the respiratory pressure changes are about the same magnitude in the arteries and in the intrathoracic cavities. In other records (fig. 7) the blood pressure changes are much greater than those in the intrathoracic pressure, and are less closely in phase with them. Under some circumstance, therefore, changes of cardiac filling and blood flow are important in relation to the respiratory waves in the systemic pressure.

The next two figures (8, 9) demonstrate the effect of an increase in intrathoracic pressure upon the total (middle curves) and net (lower curves) pressures in the pulmonary artery (fig. 8) and pulmonary vein (fig. 9). The upper curve in each figure records the carotid pressure. The total pressure in both artery and vein rises when the intrathoracic pressure is increased by applying positive pressure to the trachea. The net pressure being unsupported by the increased intrathoracic pressure falls because inflow to the heart has been hindered by the increased intrathoracic pressure. There is less blood in the lungs exerting pressure on the walls of the vessels of the lungs. There is also a diminution of the systemic arterial pressure (upper curves). It is to be noted that there is a definite lag in both the rise and fall of the pulmonary venous pressure and the systemic arterial pressure as compared with the pulmonary arterial pressure. This is undoubtedly caused by the reservoir function of the lungs (11).

Experiments in which pulmonary arterial pressure is balanced against the pulmonary venous pressure. Figure 10 shows the pulmonary arterial pressure above, the pulmonary venous pressure below, and the differential pressure between. The records were taken through angiostomy cannulae (see above) from an animal that was not anesthetized. The strip on the left came from a dog two days after the operation, the strip on the right from the same dog about two weeks later. The pressures inscribed by all three records were essentially unchanged during the interval, the change in height of the differential pressure record and the small pressure waves in the arterial record on the right being artifacts.

In taking these records one distinct difficulty was encountered. As the needle was thrust into the cannula large pressure fluctuations were transmitted to the manometer by the debris within the cannula and from arterial and other impacts upon the cannula. When recorded by an adequate manometer these impact pressure fluctuations presented bizarre outlines. Inspection of the record after a strip had been developed enabled us to



Figs. 9-14

discard records where we could not recognize clearly typical arterial and venous pressure pulse contours and thus to assure ourselves that satisfactory entrance had been made into the vessels concerned.

The contour of the venous pressure pulse is essentially the same as in the anesthetized (3) dog, the A, C and V waves being clearly evident. The second cycle of the right hand strip is of ectopic origin with the A wave following the C wave. In five unanesthetized dogs the venous pressure taken during quiet breathing varied, during a cardiac cycle, between −1 and 15 mm. Hg.

The arterial pressure pulse contour in the unanesthetized animal differs in some important respects from that of the operated anesthetized animal (3). The heart is slower and better filled so that relatively high pressures are maintained throughout systole. The incisura is more marked. Diastole is often so long that pressure descent almost ceases. The curve becomes nearly horizontal and is not of the same mathematical type as

Fig. 9. Upper curve, carotid pressure. Middle curve, pulmonary venous pressure. Lower curve, pulmonary venous pressure minus intrathoracic pressure. In the middle of the record the lungs are suddenly inflated with air under positive pressure. Time, 10 seconds.

Fig. 10. Unanesthetized dog. Upper curve, pulmonary arterial pressure (42/11 mm.Hg). Lower curve, pulmonary venous pressure (2–12 mm.Hg). Middle curve (retouched), pulmonary arterial minus pulmonary venous pressure (30/1 mm.Hg). Left record, 2 days after operation. Right record, 2 weeks later. Time, 1 second. Nine months later, when this paper was in proof, this same dog, with the arterial cannula still in place, gave pulmonary arterial pressure curves indistinguishable from those presented here.

Fig. 11. Upper curve, carotid. Second, pulmonary arterial minus pulmonary venous (retouched). Third curve, pulmonary venous. Fourth curve, pulmonary arterial. In the middle of the record the lungs were inflated with air under positive pressure. Time, same as fig. 9.

Fig. 12. The effect of air emboli on the lesser circulation of an anesthetized dog. Upper curve, carotid pressure. Second curve, pulmonary arterial pressure minus pulmonary venous pressure. Third curve, pulmonary venous pressure. Fourth curve, pulmonary arterial pressure. Time, 10 seconds.

Fig. 13. (Above) The effect of 1 pearl (0.3 cc.) of amyl nitrite on the lesser circulation of unanesthetized dog. Top curve, pulmonary arterial pressure. Middle curve, pulmonary arterial minus pulmonary venous pressure (retouched). Bottom curve, pulmonary venous pressure. Time, 10 seconds.

Fig. 13. (Below) The effect of 0.01 mgm. of acetyl-beta-methylcholine chloride intravenously on the lesser circulation of an unanesthetized dog. Curves as in figure 13 (above).

Fig. 14. (Above) The effect of 0.1 mgm. epinephrine HCl intravenously upon the lesser circulation of an unanesthetized dog. Curves as in figure 13. Time, 10 seconds. Blank space denotes elapse of 30 seconds.

Fig. 14. (Below) The effect of 0.5 mgm. epinephrine HCl intravenously upon the lesser circulation of an unanesthetized dog. Curves as in figure 13. Blank space denotes lapse of 30 seconds.

the diastolic pressure curve of the systemic arteries (10). In six unanesthetized dogs the pulmonary arterial pressure was found to be between 45/12 and 28/7 and to average 37/10. The mean pressure (integrated) averaged about 20 mm. Hg. To the figures for venous and arterial pressure, 5 to 10 mm. Hg. should be added to give the effective pressure acting upon these vessels in the thorax.

We are unable to account for the decided disagreement between our results and those of Daly (9) who reports mean values of 30 to 50 mm. Hg with large pressure variations from time to time. Our dogs were in good condition. We took pains to measure only records where simultaneous venous, arterial and differential curves indicated that the needles were clear and that intrathoracic pressure was at a normal resting value. When the dog is vocalizing or straining against a closed glottis, mean pressures twice normal may easily be recorded and, if the manometer is recording extravascular impacts instead of intravascular pressures, the figures are variable and often high.

The differential pressure curve which appears between the arterial and venous curves in figure 10 is the resultant of the algebraic summation of the arterial (+) and venous (−) curves. Every rise in the arterial curve appears as a rise in the differential curve and every rise in the venous curve appears as a depression in the differential curve. This differential pressure measures the gradient of pressure between artery and vein and has an average value of 30/1 mm. Hg. It can be expected to change only when changes occur in the rate of flow through the pulmonary bed or in the degree of constriction of the pulmonary arterioles. Fluctuations due directly to intrathoracic pressure changes are canceled just as they are in the differential records already described.

It should be emphasized that there are little or no respiratory fluctuations in the pressure gradient through the lungs. This is illustrated in figure 13 (above) where, in spite of violent respiration and large respiratory changes in both arterial and venous pressures, the pressure gradient, as measured by the differential record, shows no definite respiratory fluctuations.

Intrathoracic pressure changes, however, when they are extreme and long lasting, do produce very definite changes in the gradient of pressure through the lungs by changes in the rate of flow. In figure 11 the intrathoracic pressure was suddenly elevated (anesthetized dog acute experiment). This resulted in an acute fall of the systemic arterial pressure (top record) and a rise of both the pulmonary arterial (bottom record) and the pulmonary venous pressure (3rd record). The record next to the top is the differential pressure. As soon as the positive intrathoracic pressure begins to interfere with blood flow into the thorax the pressure gradient forcing blood through the lungs drops and remains down until

the positive pressure is released. Then blood rushing into the thorax through the great veins is pumped into the pulmonary artery whence it flows into the pulmonary vein, producing a large but very temporary rise in the gradient of pressure through the lungs which definitely precedes the rise in the carotid pressure.

In the acute experiments it was found impossible to produce an effective change in the pulmonary peripheral resistance by physiological or pharmacological means. Recourse therefore was had to partially blocking the pulmonary bed with air embolism. Figure 12 shows a slight fall in carotid pressure (upper record), a definite rise in pulmonary arterial pressure (bottom record) while pulmonary venous pressure remains unchanged. The differential pressure shows that there has been a definite increase in the pulmonary peripheral resistance as a result of the air embolism.

Figures 13 and 14 show the effect of certain drugs on the unanesthetized animal completely recovered from the operation. In all of these the upper record is from the pulmonary artery, the lower record from the pulmonary vein, and the middle record from the differential manometer.

Figure 13 (above) illustrates the effect of amyl nitrite upon the pulmonary circulation. The rise in arterial and venous pressures is confined to expiration and is due to increased intrathoracic pressure resulting from dyspnea and vocalization produced by the irritating fumes. The mean differential pressure rises from about 15 mm. Hg to about 17 mm. Hg. It has been shown elsewhere (11) that amyl nitrite produces a marked increase in the cardiac output. The fact that despite this increase in blood flow, there is only 2 mm. Hg increase in the pressure gradient which causes the blood flow through the lungs, is clear evidence that there is a very capacious vascular bed in the lungs. The increase in blood flow is secondary to the effect of the drug upon the systemic circulation. The drug was in contact with the lung before these effects could have come about. The absence of early effects upon the pressure gradient indicates that the drug has no physiologically effective direct action upon the pulmonary resistance.

Figure 13 (below) illustrates the same points in relation to the injection of acetylcholine. As in the anesthetized animal (3) the drug produces a marked slowing of the heart, a temporary cessation of blood flow through the lungs as indicated by the fact that the differential pressure comes down to zero. During the acceleration-phase there is a very large increase above normal in the cardiac output (12) but the pressure gradient forcing blood through the lungs remains at a low level. There is no evidence of effective vasomotor responses even during the first passage of the drug in high concentration through the lungs immediately after injection (compare 13).

In figure 14 (above) 0.1 mgm. of epinephrine HCl has been injected into

a vein. The heart is slowed reflexly by the usual rise in systemic pressure. The pulse pressure in the pulmonary artery is increased but the mean pressure remains unchanged. The venous pulse gives evidence of auricular flutter and three or four to one block. There is no definite change in mean venous pressure. The differential pressure is constant throughout the epinephrine response. There can therefore be no effective vasoconstriction even during the first passage through the lungs of the highly concentrated drug.

In figure 14 (below) a very large dose of epinephrine was administered. It produced such intense systemic vasoconstriction that the left ventricle was decompensated (11). Observation of the heart in the open chest shows that the left ventricle becomes very greatly dilated as a result of such epinephrine injections. The pulmonary arterial and venous pressures rise at exactly the same time and to the same extent. This is evidently due to back pressure from the failure of the left ventricle to eject an adequate amount of blood against the high systemic pressure. The differential pressure remained constant throughout the response, even during the first passage of the drug through the lungs. There can therefore be no effective vasoconstriction in the pulmonary bed in response to epinephrine—or in fact in response to any other maneuver which we have tried.

In the anesthetized animal in acute experiments we have observed a definite though slight fall in the differential arteriovenous pressure in spite of a large rise in both the arterial and venous pressures. The fall is probably due to a diminution of blood flow from the large injection of epinephrine (11, and earlier workers cited here) and not (for reasons given above) to a vasodilatation in response to the drug.

Aid from the American Medical Association in carrying out this investigation is gratefully acknowledged.

SUMMARY

A modification of the London technique for placing angiostomy cannulae upon the pulmonary vessels, an advantageous device for administering artificial respiration and the technique for making optical tracings of the pressures in the pulmonary artery and vein of unanesthetized dogs are described.

By means of differential and ordinary "hypodermic" manometers, records were made of the pressures in the pulmonary artery, and pulmonary vein, of the effective pressures distending these vessels within the thorax and of the gradient of pressure forcing blood through the lungs.

In normal unanesthetized dogs, breathing quietly, the pulmonary arterial pressure varies between 45/12 and 28/7 mm. Hg in different dogs and averages 37/10. The mean pressure (integrated) averages about

20 mm. Hg. The pulmonary venous pressure, taken during quiet breathing, averages 3 to 12 mm. Hg.

Inspiration lowers total pressure in both the systemic and pulmonary arteries, but raises slightly the effective pressure in the pulmonary artery. The total pressure in the pulmonary vein is lowered by inspiration but the effective pressure is changed very little. The expiratory increase in systemic arterial pressure is caused partly by an increase in intrathoracic pressure and partly by an increase in cardiac output.

The gradient of pressure forcing blood through the lungs is decreased by a prolonged rise in intrathoracic pressure and increased immediately afterwards. It is also increased by air embolism. It is unaffected by ordinary respiration. Vasomotor drugs have no direct effect upon this gradient. Secondary effects due to changes in blood flow are so small as to imply very definitely that the pulmonary channels are quite capacious.

The rise in pulmonary arterial pressure after large doses of epinephrine is due to back pressure from the left ventricle and is not accompanied by an increase in the gradient of pressure from artery to vein. We can supply no clear cut evidence that vasoconstriction in the pulmonary bed plays any significant rôle in the dynamics of the lesser circulation.

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SIMULTANEOUS DETERMINATION OF THE PLASMA VOLUME WITH T-1824,[1] AND THE "AVAILABLE FLUID" VOLUME WITH SODIUM THIOCYANATE[2]

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Considerable progress has been made in recent years in devising and perfecting dilution-methods for estimating the quantity of water in the three fluid compartments of the body in intact animals and man. Certain colloids (e.g., gum acacia (2), hemoglobin (3), anti-crystallized-egg-albumen serum (4), diphtheria anti-toxin (5)), and vital dyes (e.g., vital red (6), T-1824 (7)), leave the blood stream so slowly that for practical purposes it may be assumed that after being injected they remain in the plasma compartment during the time required for uniform mixing. Consequently, the plasma volume may be estimated by observing the extent to which a measured amount of one of these substances is diluted by the circulating blood. There is now evidence (8, 9, 10) that one can also measure, at least approximately, the total extracellular fluid volume with the thiocyanate-dilution method introduced by Crandall and Anderson (11); and quite recently, Painter (12) has found that in dogs the water available for the solution of either urea or sulfanilamide is in all probability equivalent to the total body water. It is apparent that these measurements would be of greater usefulness if they could be made simultaneously. The authors, therefore, have tried to combine the determination of plasma volume, utilizing T-1824, with the determination of the fluid available for the solution of thiocyanate. The present paper includes the results of preliminary tests, a brief description of the technic for measuring plasma volume and "available fluid" at one time, and a summary of data obtained on normal dogs.

[1] Recent attempts to rename this dye "Evans Blue" are to be deplored. Various authors, including Dr. H. M. Evans (1), have for many years referred to it in the literature as T-1824. To avoid confusion this term should be retained, particularly since it is based on the structure of the dye.

[2] Aided by grants from the Committee on Grants-in-Aid, National Research Council and The Rockefeller Foundation.

METHODS. *Determination of T-1824 and thiocyanate.* The plasma concentration of T-1824 was determined by spectrophotometric analysis of serum (König-Martens spectrophotometer) at 620 mμ. Further details may be found in previous papers (13, 14, 15). The problem to be considered here was whether or not the spectral absorption of T-1824 in plasma is altered by thiocyanate. In concentrations exceeding by two or three times that required for estimating "available fluid," thiocyanate has no effect on the optical density of a standard dye solution (0.002 per cent) in serum.

Thiocyanate was determined as ferric thiocyanate according to the directions of Crandall and Anderson (11), except that the spectrophotometer was used in preference to a colorimeter for measuring the color reaction. Spectrophotometric technic has two important advantages: it permits the analysis of smaller samples (0.5 cc. serum), and is more accurate, especially in repeated determinations of "available fluid" when the blood contains residual thiocyanate (13). Although the peak of the absorption curve of ferric thiocyanate falls at 460 mμ (see fig. 1), it was found after a number of trials that spectrophotometric readings were usually more consistent and could be carried out with less eyestrain at somewhat longer wave lengths. All determinations reported here have been based on readings at 480 mμ. Some precaution must be taken to prevent fading after ferric nitrate has been added. If the samples are kept in diffuse or subdued light, there is no change in the optical density for several hours. It should be noted, in passing, that untreated serum may be kept in the ice box for several days, or even a month, without loss of thiocyanate. Serum in which the proteins have been precipitated with trichloroacetic acid likewise remain stable for some time. Samples treated with the acid and left standing for as long as 40 hours before the ferric nitrate is added still yield the same values for thiocyanate as when analyzed immediately after the precipitation of the proteins. The relation between the optical density (10 mm. depth) of ferric thiocyanate and the concentration of thiocyanate expressed as milligrams per cent of the sodium salt is shown in figure 2.

From figure 1 it may be seen that a direct analysis for thiocyanate would be inaccurate in a sample containing T-1824 unless the dye is completely removed from solution prior to the addition of ferric nitrate. In the analysis of serum, this problem offers no difficulty since the dye comes down with the protein precipitate. Even if the protein concentration is greatly reduced (1–2 per cent) the solution is still cleared of dye by adding trichloracetic acid. The acid does not, however, precipitate T-1824 in a protein-free solution.

Directions for measuring plasma volume and "available fluid" in the dog or in man. For convenience, sterile solutions of T-1824 (1 per cent in water[3]) and sodium thiocyanate (5 per cent) should be prepared in rather large batches and kept respectively in 5 and 20 cc. sealed glass ampules.[4] Under these conditions the solutions remain stable indefinitely and need only be standardized once. Two cubic centimeters of the dye are adequate for determining plasma volume in a human subject of average size provided the serum samples are read in 20 mm. absorption cells (capacity 1 cc.) (14). This amount causes no visible staining. For dogs it is advantageous to use somewhat more dye (0.05–0.1 cc. per kgm. body weight) so that the serum samples may be read in 5 or 10 mm. absorption cells. Approximately 20 mgm. of sodium thiocyanate per kilogram should be given in order to obtain a plasma concentration that can be determined readily (10 mgm. per cent).

Four cubic centimeters of blood are drawn without stasis from one antecubital

[3] T-1824 is not stable in saline unless some protein is also present.

[4] The solutions were prepared by Hynson, Wescott and Dunning, Baltimore.

vein (in dogs the jugular is used) and placed under oil. The needle is left in the vein. T-1824 is then injected from an accurately calibrated syringe, the syringe being cleared of all the dye by rinsing repeatedly with blood. This is followed immediately by the injection, through the same needle, of the 5 per cent sodium thiocyanate; the thiocyanate syringe is also rinsed carefully with blood and the needle then removed. Every twenty minutes for two hours following the injection, a blood sample (3 cc.) is collected from the opposite antecubital vein. The subject should remain quiet (lying down) throughout this period. The blood samples are permitted to clot under oil, centrifuged, and the supernatant serum collected. It is essential to centrifuge the samples a second time in order to free them completely from cells and oil droplets.

By reading the dye-tinged samples directly against the control sample the absorption by the serum itself is cancelled (14). The optical densities of T-1824 in the dye

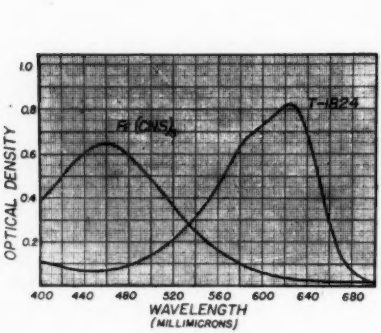

Fig. 1

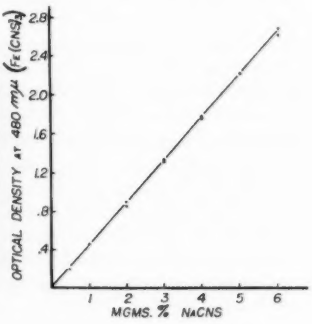

Fig. 2

Fig. 1. Spectral absorption curves (Hardy recording spectrophotometer) of ferric thiocyanate (equivalent to 1.25 mgm. per cent NaCNS) and of T-1824 (0.001 per cent in serum).

Fig. 2. Showing the relation between the optical density (10 mm. cell) of ferric thiocyanate at 480 mμ and the concentration of thiocyanate in terms of milligrams per cent sodium thiocyanate. Data obtained from analyses of standard solutions made up in serum.

samples are then plotted as a time-concentration curve (disappearance curve) which, except at the start, is practically a straight line (see fig. 3). The optical density which is obtained by projecting this line back to the time of injection is assumed to represent the dye-concentration which would exist if the dye were uniformly distributed in the plasma and none of it had been lost from the circulation. From this value, D_p, and from the optical density of the standard one per cent T-1824 in known dilution (1–500), D_k, the plasma volume may then be calculated as follows:

$$\text{Plasma volume cc.} = \frac{D_k \times 500 \times \text{cc. dye injected}}{D_p}$$

One-half cubic centimeter of serum is removed from each sample for the thiocyanate analyses. The control must be treated in exactly the same manner as the rest, regardless of whether or not it contains thiocyanate, in order to provide a suitable blank against which the unknowns can be read in the spectrophotometer.

Optical density is converted into milligrams per cent sodium thiocyanate by referring to figure 2, and by correcting for the dilution of the sample during analysis. The results of typical experiments are plotted in figure 3. After 30 or 40 minutes following the injection, there is usually no change in the plasma concentration of thiocyanate for several hours. Hence the excretion of this substance is apparently slow enough to obviate the necessity of correcting for the amount lost during the mixing period. Nevertheless, it is not safe to base the determination of "available fluid" upon the analysis of a single sample, for occasionally the time-concentration curve does not reach a plateau, but continues to fall quite rapidly throughout the period of observation. If one assumes that the thiocyanate is distributed in the same con-

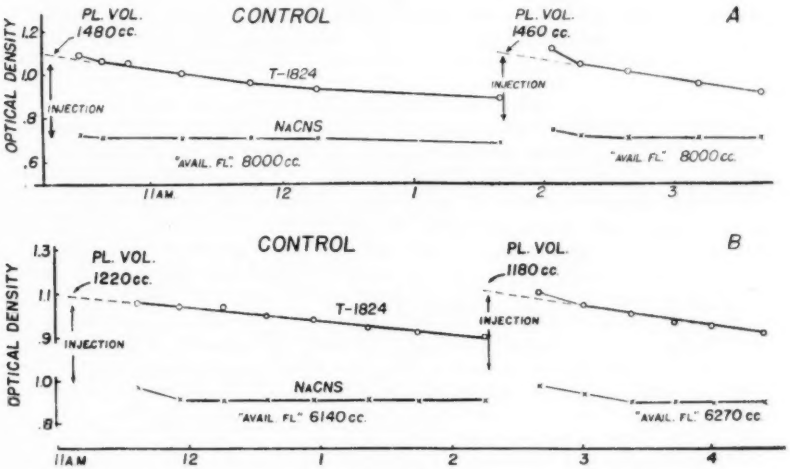

Fig. 3. Repeated determinations of plasma volume and "available fluid" showing the graphic method used in correcting for the dye lost during the period of "mixing." The samples obtained after the second injection are read against the "control" sample taken immediately prior to this injection.

A. Sept. 23, 1935. Male police dog. Body weight 31.0 kgm. Dye: 2.024 cc. 1 per cent T-1824. Thiocyanate: 10.126 cc. 5 per cent NaCNS.

B. Feb. 11, 1938. Male collie dog. Body weight 23.6 kgm. Dye: 1.639 cc. 1 per cent T-1824. Thiocyanate: 10.124 cc. 5 per cent NaCNS.

centration throughout all the fluid in the body that contributes to its dilution, the "available fluid" may be calculated from the equation:

$$\text{"available fluid" cc.} = \frac{\text{CNS injected (mgm.)} \times 100}{C_p \text{ (mgm. per cent)}}$$

in which C_p is the plasma concentration when equilibrium is reached.

How closely the "available fluid" volume corresponds to the total extracellular fluid volume is still undetermined. Crandall and Anderson (11) found that thiocyanate enters the erythrocytes. The present authors have repeated their experiments and confirmed their results on the distribution of thiocyanate between the erythrocytes and plasma. In view of the fact that sodium thiocyanate cannot be

recovered quantitatively as ferric thiocyanate from hemoglobin solutions, the question arose as to whether or not thiocyanate combines with hemoglobin inside the red blood cells. This is apparently not the case, since in whole blood containing thiocyanate, displacement of the plasma with Ringer's solution causes the thiocyanate remaining in the red blood cells to be distributed equally throughout all the fluid in the system. The "available fluid" inside the erythrocytes is therefore equal to their water content (about 70 per cent) multiplied by the total red cell volume. The latter is often estimated from the total plasma volume and the relative cell volume (hematocrit). It should be emphasized that the figure so obtained is incorrect since the blood in the smaller vessels and capillaries contains fewer cells per unit volume than that in the larger vessels (16). Direct determinations of the total red cell volume with the carbon monoxide method are 25 to 30 per cent lower than the values calculated indirectly from the plasma volume (dye-method) and the hematocrit(17). If the latter method is used, the figures should be multiplied by a factor of approximately 0.75.

Lavietes and his co-workers (8) have suggested that a value more nearly representing the extracellular fluid is derived by including a correction factor based on their observations that the concentration of sodium thiocyanate in serum is about 10 per cent higher than in ultrafiltrates of serum. It may be pointed out, however, that the possible error incurred by neglecting an unequal distribution of thiocyanate between plasma and interstitial fluid is offset to an unknown extent by the amount of thiocyanate which undoubtedly enters the cells of certain glandular organs (e.g., pancreas, salivary glands). Indeed the problem of deciding exactly what corrections should be applied to the "available fluid" determination is complicated further by the fact that in the body as a whole sodium and chloride are not exclusively extracellular, and therefore, the extracellular fluid compartment, defined anatomically, may not actually correspond to the space which functions as such. In view of these considerations the results obtained with the thiocyanate method in the following experiments have been reported simply as "available fluid."

Results. Data on man will not be presented in this paper. Representative results on dogs are included here to show: 1, the agreement which is obtained when the determinations are repeated under the same conditions within the space of a few hours; 2, the range of individual differences in the plasma volume and "available fluid" per kilogram body weight; and 3, the variations in results obtained on the same dogs studied over a period of months.

The data plotted in figure 3 were secured on two well-trained dogs which would lie quietly for many hours quite undisturbed by the veno-punctures. Under such favorable experimental conditions, it is not unusual to find that successive control determinations agree as well as those shown in figure 3. Hence, any marked changes in plasma and "available fluid" volumes can be detected readily. This is illustrated by the observations presented in table 1 which were made on the first of the two dogs just mentioned (see fig. 3 A). The animal was given 900 cc. of 50 per cent sucrose slowly by vein. In 7 hours the kidneys had excreted 1600 cc. of fluid in excess of the amount injected, and the body weight had dropped 1.93 kgm. From a comparison of the plasma and "available fluid"

volumes at this time with the control determinations in figure 3 A, it may be inferred that more than one-half of the water excreted had been drawn from the intracellular phase. The dog was now given water *ad libitum*, but no food. By the following morning, the body weight had increased 1.4 kgm. It is interesting to observe that water alone restored both plasma volume and "available fluid" volume to the normal levels shown in figure 3 A.

The simultaneously determined values for plasma volume and "available fluid" from 73 control experiments on 15 normal unanesthetized dogs are listed in table 2. The animals were given water *ad libitum*, but no food for 18 to 24 hours prior to the tests. This precaution is necessary in order to

TABLE 1

The effect of water ingestion on the plasma volume and "available fluid" volume of a dog dehydrated by the intravenous injection of hypertonic sucrose

	BODY WEIGHT	PLASMA VOLUME	"AVAILABLE FLUID"	INTERSTITIAL FLUID* (CALCULATED)
	kgm.	*cc.*	*cc.*	*cc.*
Oct. 29, 1935:				
10:00 a.m.	30.13			
10:00 a.m.–3:00 p.m.	900 cc. 50 per cent sucrose i.v.			Body water excreted = 1600 cc.
10:00 a.m.–5:00 p.m.	2500 cc. urine collected			
5:00 p.m.	28.2	1260	7320	5660
Water ad libitum (no food)				
Oct. 30, 1935:				
10:00 a.m.	29.6	1485	8250	6360
Change	+1.4	+225	+930	+700

* These figures for interstitial fluid were calculated by merely subtracting the plasma volume and the quantity of fluid in the red blood cells (estimated to be 400 cc.) from the total "available fluid."

avoid errors from lipemia (13) and to eliminate the disturbing effects of digestion (18) upon the plasma volume. At least 30 minutes before the start of a determination the dog was placed on its back on a well-padded animal board where it lay quietly throughout the duration of the experiment, usually $2\frac{1}{2}$ to 3 hours. The thongs, when used, were arranged so that they could not interfere with the blood flow through the extremities. With the animal in this position, blood may be drawn *without stasis* from the external jugular veins. About 8 samples were collected in each experiment and the results plotted for calculation of plasma volume and "available fluid" as shown in figure 3. Except for the control, the samples were all taken from the vein opposite the site of injection of dye and thiocyanate.

TABLE 2

Numbers in italics following averages represent number of determinations on which average is based.

DOG NO.	DATE	BODY WEIGHT	PLASMA VOLUME		"AVAILABLE FLUID"	
			Cc. per kgm.	Average	Cc. per kgm.	Average
		kgm.				
1	1/20/37– 6/15/37	16.5–17.0	44.8–53.1	48.4(*6*)	238–256	248(*6*)
Male mongrel (6 yrs.)						
2	1/11/37– 2/14/38	19.0–20.1	46.5–57.9	51.9(*6*)	250–284	263(*6*)
Male airedale (8 yrs.)						
3	1/25/37	23.2	50.6		266	
Male collie	2/ 8/37	24.0	46.6		266	
(7 yrs.)	2/15/37	25.4	44.9		248	
	3/ 2/37	25.7	43.6	48.1	252	258
	6/10/37	26.4	45.0		248	
	2/11/38	23.6	51.4		260	
			51.0*		266	
	3/ 7/38	23.5	51.7		255	
4	2/ 5/37	20.25	59.0		283	
Male mongrel	2/11/37	20.35	60.0	57.0	291	280
(8 yrs.)	7/27/37	20.2	59.0		292	
	2/14/38	23.1	50.0		254	
5	1/ 5/37	10.8	56.0		284	
Male mongrel	1/19/37	10.3	64.0	58.2	315	295
(full-grown,	4/ 2/37	10.2	61.9		304	
age unknown)	8/ 3/37	12.0	50.9		276	
6	11/10/36– 1/22/37	12.5–13.3	59.0–66.0	61.0(*4*)	333–350	342(*4*)
Male mongrel (young)						
7	6/25/37– 9/22/37	18.2–20.4	62.0–65.4	63.7(*2*)	369–426	398(*2*)
Male police dog (8 mo.)						
8	3/25/37– 5/25/37	9.7–12.1	63.0–64.5	63.8(*2*)	341–354	348(*2*)
Male mongrel (young)						
9	1/ 9/36	9.9	54.5		372	
Female mongrel	7/ 6/36	10.5	56.0		368	
(6 mo.)	7/ 9/36	10.1	57.8	58.1	407	384
	7/16/36	10.3			391	
	7/31/36	10.2	64.1		380	
	12/ 9/36	13.6	52.9		314	
	12/11/36	13.35	53.6		297	
	6/ 8/37	13.9	47.4	49.2	270	292
	1/24/38	13.6			280	
	2/ 2/38	13.7	43.0		285	

* Corrected for plasma removed during the first determination.

TABLE 2—*Concluded*

DOG NO.	DATE	BODY WEIGHT	PLASMA VOLUME		"AVAILABLE FLUID"	
			Cc. per kgm.	Average	Cc. per kgm.	Average
		kgm.				
10	12/ 8/36	9.5	58.9	59.2	362	352
Female beagle	1/15/37	10.5	59.4		342	
(8–10 mo.)	6/28/37	12.3	48.6	47.6	267	276
	7/ 1/37	12.35	46.2		269	
	7/15/37	11.9	50.6		291	
	3/ 4/38	12.4	45.2			
11	12/ 9/36– 1/ 7/37	9.7 –10.7	52.5–55.1	53.8(2)	335–374	355(2)
Female beagle	2/26/37– 1/28/38	13.17–13.4	40.0–48.5	44.3(2)	260–278	269(2)
(10 mo.)						
12	12/ 8/36– 1/ 7/37	10.2 –10.7	54.2–61.9	58.1(2)	336–373	355(2)
Female beagle	2/ 5/37– 7/ 1/37	12.4 –12.6	50.0–52.6	51.6(3)	291–298	294(3)
(10 mo.)						
13	1/ 4/37– 7/15/37	15.4 –16.7	49.1–59.4	54.3(4)	255–318	293(4)
Female mongrel						
(full-grown)						
14	11/ 5/36– 7/26/37	12.5 –13.3	53.0–62.1	57.2(5)	282–318	295(5)
Female mongrel						
(full-grown)						
15	6/10/37–11/18/37	11.7 –13.0	53.5–56.5	54.6(4)	282–312	298(4)
Female mongrel						
(full-grown)						

Dogs show large individual differences in plasma volume and "available fluid" per kgm. body weight (table 2). In normal animals obtained from the laboratory supply during the past three years the plasma volume has been found to range from 35 to 65 cc. per kgm. and the "available fluid" from 230 to 425 cc. per kgm. "Average normal" values derived from the study of a large group in which there is no uniformity with respect to age, sex or breed are therefore of relatively little value.

Most of the dogs listed in table 2 were under observation for periods of several months. In comparing the results obtained on the same dog at different times, the reader should bear in mind that these animals were given only the routine laboratory care.[5] No attempt was made to control the water balance either by regulating the food and salt intake, or by adjusting the temperature and humidity of the environment. And yet, the results of repeated determinations, at least on the adult males, have usually been quite consistent provided the body weight remained approximately the same. In adult female dogs there seems to be less regularity in the results, even if one excludes the values obtained during pregnancy or

[5] The diet consisted almost entirely of Beacon dog pellets.

when the dogs are in heat. The data on dogs 3, 4 and 5 illustrate the effect of marked variations in weight caused by changes in body fat. Although the total volumes do not change materially, the values in cc. per kgm. fall as the body weight increases. In comparison with the rest of the body, fatty tissue contains very little water (20 to 30 per cent). Changes in degree of adiposity are therefore reflected in the per cent total body water (19) as well as in the plasma volume (20) and "available fluid" (11) per unit body weight.

It has long been recognized that the per cent total body water is higher in young individuals than in adults. That this holds also for plasma volume and "available fluid" expressed in cubic centimeters per kilogram is indicated by a comparison of the average values on dogs 1, 2, 3 and 4 (6–8 years old) with those on 6, 7 and 8 (less than 1 year old). The change with age is convincingly demonstrated by the series of determinations on the female dogs 9, 10, 11 and 12 on which the observations were begun when the animals were considerably less than 1 year old and still growing. The results obtained on each of these dogs up to the time it was about 1 year old have been averaged for comparison with those collected after it had reached full size. It is quite evident that as the animals matured there was a marked drop in both plasma volume and "available fluid" per kgm. It may also be seen that the average values for "available fluid" at the beginning (352–384 cc. per kgm.) are roughly the same as in the young males 7, 8 and 9 (342–398 cc. per kgm.), whereas the subsequent values (269–294 cc. per kgm.) are comparable with those obtained on the adult males 1, 2, 3, 4, and 5 (248–295 cc. per kgm.) and on the adult females 13, 14 and 15 (293–298 cc. per kgm.). The plasma volume data do not fall into such distinct categories according to the ages of the dogs, primarily because the individual differences in plasma volume among dogs of the same age group are so large relative to the general change.

One of the purposes in determining plasma volume and "available fluid" simultaneously was to ascertain whether or not there is normally some definite relation between the plasma volume and the total extracellular fluid volume. Gamble (21, 22) has in recent years expressed the belief that when the total fluid balance undergoes a change, the plasma volume is held at its normal level by adjustments in the interstitial compartment. His concept that the interstitial fluid serves the plasma in a sustaining capacity cannot of course be questioned, but work now in progress does not indicate that the volume of the plasma is independent of the quantity of interstitial fluid even with moderate changes in the total volume of extracellular fluid. Furthermore, it is apparent from the results listed in table 2 and from the preceding discussion that there is at least a general parallel between the values for plasma and "available fluid" in normal dogs. The relationship is shown graphically in figure 4 A, which

has been constructed from the data on the male dogs by plotting the values for cc. of plasma per kgm. against the simultaneously determined "available fluid" in cubic centimeters per kgm. A similar treatment of the results on the females is presented in figure 4 B. The data in these two graphs evidently show that the ratio of $\frac{\text{"available fluid"}}{\text{plasma volume}}$ is greater in animals in which the volumes per kilogram are high. They also indicate that the females have more "available fluid" relative to plasma than the males. This difference between the sexes should be confirmed by observations on litter mates, or at least on dogs of the same breed.

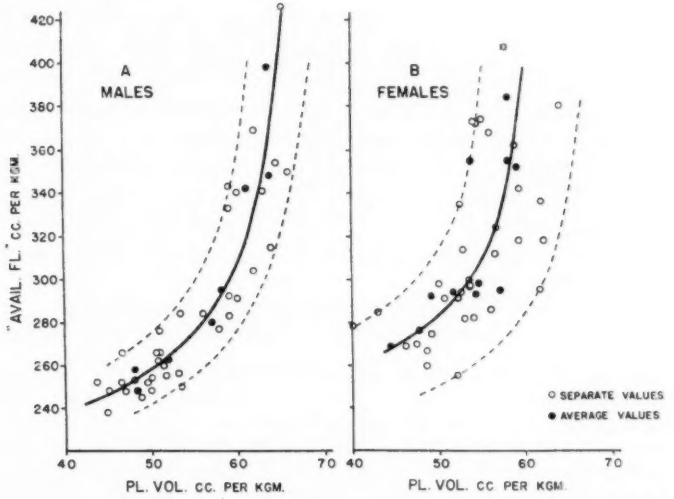

Fig. 4. Showing the relation between plasma volume and "available fluid" in normal dogs—A, males; B, females.

SUMMARY

Directions for carrying out simultaneous determinations of plasma volume with the blue dye, T-1824, and of "available fluid" with thiocyanate are briefly outlined. The results of preliminary tests show that these methods may be combined without affecting the accuracy of either the T-1824 or the thiocyanate analysis. By determining the thiocyanate (as ferric thiocyanate) as well as the dye concentration with a spectrophotometer (see fig. 1), both analyses may be completed on 0.5 cc. of serum. Spectrophotometric technic, furthermore, eliminates the errors encountered with colorimeters (13) when the plasma volume and "available fluid" determinations are repeated and the blood contains "residual" dye or thiocyanate (fig. 3).

From the results of simultaneous determinations of plasma volume and "available fluid" in dogs (table 2), it may be stated that 1, in this species there are large individual differences in both plasma volume and "available fluid" per kilogram body weight; 2, under normal conditions the plasma volume is a function of the total volume of "available fluid" (fig. 4, A and B); 3, as the animals mature, they show a striking decrease in the volume of plasma and "available fluid" per kilogram body weight (dogs 9, 10, 11 and 12, table 2) and that 4, females appear to have more "available fluid" relative to plasma than males (cf. fig. 4, A and B).

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CERVICAL SYMPATHETIC STIMULATION AND BASAL METABOLISM

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The functional innervation of the thyroid gland has been the subject of an extensive controversial literature. The details of mutually contradictory reports have been reviewed too often to warrant repetition. Suffice it to state that there is no clear-cut, direct or undisputed evidence that the rate of secretion of thyroglobulin can be influenced through the nervous connections of the thyroid gland. Nevertheless, many investigators have persisted in this belief, principally because of the profound disturbances of the autonomic nervous system which are characteristic of or associated with the hyperthyroidism of exophthalmic goiter. In one of the more recent attempts to establish the autonomic control of thyroid function it was reported (Haney, 1932) that faradic stimulation of the rabbit's cervical sympathetic trunk for 1 to 3 hours resulted in an extraordinary increase in basal metabolic rate which persisted over a period of 3 to 6 weeks. Haney concluded that this increase in metabolism was due to an acceleration of the functional activity of the thyroid gland. It was obviously important to repeat these observations.

METHOD. Twenty-four young but sexually mature rabbits (23 females and 1 male) were isolated individually in roomy cages for a period of 2 weeks prior to experimentation. They were all fed on a diet of fresh vegetables (carrots, spinach, lettuce) and a dry food mixture of alfalfa hay and oats. The environmental temperature was usually 28°C., although no attempt was made to regulate this with precision. Their basal metabolic rates (calories per kilogram per hour) were determined repeatedly under basal conditions at 28°C. in a modified Benedict closed-circuit apparatus (Friedgood, 1934) until the animals were thoroughly accustomed to the experimental procedure. When consistent readings were obtained, 3 to 8 preliminary determinations, usually checking within ±5 per cent, were made on alternate days. The cervical sympathetic nerves were then stimulated in 22 of these rabbits by applying insulated bipolar silver-wire electrodes to the right and left trunks after tying off the nerves proximal to the electrodes. The preparation and implantation of the electrodes were modified from the original technique developed by

Bradford Cannon (1933) for the stimulation of autonomic nerves. The current was supplied by condenser discharges or through a Harvard coil and battery. The method and duration of stimulation varied considerably in individual instances, and details concerning them are recorded in table 2. The intensity of stimulation was adjusted to subject the blood vessels of the ears to maximal vasoconstriction. In most instances, the rate of stimulation was limited to 20 to 30 shocks per second, because this rate has been shown to produce an optimal physiological effect upon sympathetic nerve fibers (Bishop and Heinbecker, 1932; Knoefel and Davis, 1933; Orías, 1932; Rosenblueth, 1932). In a few cases, a tetanizing current from a Harvard coil was employed in conformity with Haney's technique.

Some of the experiments were done with ether anesthesia which was administered only during the operation, stimulation of the sympathetic nerves being performed subsequently in the unanesthetized animal. Other experiments were carried out with light urethane anesthesia (0.4 to 0.6 gram urethane per kilogram, intravenously).

In the remaining 2 animals the vagus nerve was stimulated in its mid-cervical portion by 20 to 30 condenser discharges per second. It was not tied off, and stimulation was therefore directed both centrally and peripherally. Marked slowing of the heart rate was used as evidence of adequate intensity of stimulation. In BW (table 1, group D) the left vagus was stimulated intermittently over a period of $2\frac{1}{2}$ hours with 5-minute intervals of rest after each 5 minutes of stimulation. In R21 (table 1, group D) both vagi were stimulated alternately for 15-minute intervals over a period of 2 hours.

Results. The 22 rabbits whose cervical sympathetic nerves were stimulated have been divided into 3 groups for convenience of reference. There are 12 rabbits in the first group (A, table 1). Their basal metabolic rates did not change significantly as compared with their individual basal levels determined prior to sympathetic stimulation. Eight rabbits comprise the second group (B, table 1). Their metabolic rates increased 12 to 19 per cent at one or more points in the postoperative experimental period. The basal rates of these animals, however, fluctuated irregularly and were not found at elevated levels for more than 1 or 2 determinations in contradistinction to Haney's striking observations. There were no rises in rectal temperature to account for these minor increases in metabolic rate; ordinarily one would have considered them somewhat outside the upper limit of experimental error (approximately +10 per cent). In the third group (C, table 1) are the remaining 2 animals whose rates of oxygen consumption increased considerably after cervical sympathetic stimulation. The metabolic rate of one of these animals did not begin increasing until the eighth day. Having maintained its elevated level for 5 days, it was autopsied and its thyroids were examined. The basal metabolic

rate was +33 per cent at that time, but histological examination of the thyroids revealed a normal parenchyma. The other rabbit developed a marked increase in its rate of oxygen consumption within 24 hours after cervical sympathetic stimulation. This rise was maintained for only 4 days, after which the rate of metabolism decreased rapidly to normal limits. The thyroids were not examined because the metabolic rate had already returned to normal before the experiment was terminated.

The 2 rabbits whose vagi were stimulated were followed for 4 and 13 days, respectively, after completion of the stimulation. No significant change occurred in their rates of metabolism (D, table 1).

TABLE 1

Per cent change in basal metabolic rate after cervical sympathetic stimulation

GROUP	EXPERIMENT NUMBER	DAYS OF EXPERIMENTAL PERIOD																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	6♀		+10		+4		+1		+4			−1									+1
	RN♀	+6		+9		+8			+5		+7			−2						+6	
	I3♀	+9	+3	+4	+5		+8				+4					+2					
	5♀	−4	−7	−11	−13	−18		−17	−19		−26			−26		−26					
	G7♀	+1		+6			+6			−6						0					
	3♀		+5		−2		−1			−4		+7		+4							
	7♀	+9			−3				+1			−1									
	1♀		−6	−9	−5		−6		−5												
	4♀	−9		+5		+4			+5												
	8♀		+6		−1		−1														
	9♀	−4		+1		−1															
	2♀		0	0		−3															
B	20♀	0	+10	+5				+13		+3							+5				+3
	14♀			+13	+8	0			0			+16	+13	−2		−5		−10			
	13♀		+14	+19		+2		+10		+11				+2		−5					
	17♀		+17		+13	+18		0		+6			+12			0					
	18♀			+16		+5			0												
	19♀	+10		+15	+13			0													
	16♀		+12		+3		+3														
	15♀	+13	0		+13																
C	21♀			+1		−5			+28		+27		+31	+33							
	GW♂	+26	+22		+25		+11		+9		+9										
D	BW♀		−1	−1	−4		−11		−4		−2			−5							
	R21♀	+10	+6		+6																

Discussion. The foregoing results are obviously not in accord with those published by Haney. Because of this, one of us visited Haney to find out whether there were any differences in technique which might account for the great disparity between the results of the two series of experiments.

The experimental conditions under which Haney worked differed somewhat from those in the present investigation. He used rabbits raised in Wisconsin and fed them with vegetables grown in the same locality. Our

TABLE 2

GROUP	EXPERIMENT NUMBER	DATE	METHOD OF CERVICAL SYMPATHETIC NERVE STIMULATION	RATE OF STIMULATION PER SECOND	DURATION OF STIMULATION	ANESTHESIA
					hours	
A	1 ♀ *	4/ 9/35	Both nerves simultaneously and continuously	25	½	Ether for dissection only
	2 ♀ *	4/24/35	Haney's technique		1¼	Urethane
	3 ♀ *	5/ 9/35	Haney's technique		1½	Urethane
	4 ♀ *	10/23/35	Both nerves simultaneously; 1-min. rest periods every 5 min.	25	1¾	Ether for dissection only
	5 ♀ †	2/18/36	Both nerves alternately every 1½ min.	20	2	Urethane
	6 ♀ †	1/ 7/37	Both nerves alternately every 2 min.	30	2	Urethane
	7 ♀ †	11/ 5/36	Both nerves alternately every 2 min.	20–30	2	Urethane
	8 ♀ †	1/17/36	Both nerves simultaneously and continuously	20	2	Urethane
	9 ♀ *	5/20/35	Haney's technique		2¼	Urethane
	RN ♀ †	1/ 8/37	Both nerves alternately every 2 min.	20	3	Urethane
	G7 ♀ †	1/12/37	Both nerves alternately every 2 min.	20	3	Urethane
	I3 ♀ †	2/ 8/37	Both nerves alternately every 2 min.	30	4⅙	Urethane
B	13 ♀ *	3/26/35	Both nerves simultaneously 1-min. rest period every 15 min.	25	1	Ether for dissection only
	14 ♀ *	3/29/35	Both nerves simultaneously and continuously	30	1	Ether for dissection only
	15 ♀ †	10/28/35	Both nerves simultaneously and continuously	20	1⅛	Urethane
	16 ♀ *	4/18/35	Haney's technique		1½	Ether for dissection only
	17 ♀ †	2/20/36	Both nerves simultaneously; 10-min. rest period every 30 min.	20	2½	Urethane
	18 ♀ *	5/17/35	Haney's technique (left nerve only)		3	Urethane

Source of stimuli:

* Induction coil and battery.

† Condenser discharges.

Haney's technique. Source of stimuli: induction coil and battery. The cervical sympathetic trunks were exposed on both sides, cut low in the neck, and their cephalic ends stimulated alternately with an interrupted tetanic current for 1½ minutes over a period varying from 1 to 3 hours.

TABLE 2—*Concluded*

GROUP	EXPERIMENT NUMBER	DATE	METHOD OF CERVICAL SYMPATHETIC NERVE STIMULATION	RATE OF STIMULATION PER SECOND	DURATION OF STIMULATION	ANESTHESIA
					hours	
B—*Con.*	19 ♀ †	10/25/35	Both nerves simultaneously and continuously	25	3¼	Urethane
	20 ♀ †	2/ 3/37	Both nerves alternately every 2 min.	20	4¼	Urethane
C	21 ♀ *	5/10/35	Haney's technique		2¼	Urethane
	GW ♂ †	10/27/36	Both nerves simultaneously and continuously; 15-min. rest period after 1¼ hours	20	2½	Urethane

rabbits and their food came from the environs of Boston. Wisconsin is in the goiter district; Boston is not. Since Haney did not section the thyroid glands of any of his animals there is no way of knowing whether a high percentage of them had endemic parenchymatous goiters. It is unknown, furthermore, whether such a hypertrophic thyroid would have reacted differently from a normal gland to the experimental conditions here outlined.

Haney's animals were quartered in unheated rooms, and their basal metabolic rates were determined at room temperatures. Records of the latter were not published. Our animals were quartered at 28°C., and the metabolism determinations were carried out at 28°C., because of the well-recognized effect of environmental temperature upon the rate of heat production (Rubner, 1902). In his studies with guinea pigs Rubner found that the lowest heat production was first apparent in an environmental temperature close to 30°C.; the rate of metabolism increased as the temperature was diminished progressively toward 0°C. Between 30°C. and 34.9°C. there was no appreciable change in the rate of metabolism; but there was a distinct increase in heat production as the temperature rose above 34.9°C. The range of temperature within which the rate of oxygen consumption reaches its lowest level and remains essentially unchanged has been termed the *critical temperature*. Rubner discovered, furthermore, that the critical temperature of a given animal depended in large measure on the length of hair of the animal's fur. In long-haired dogs the critical temperature was closer to 25°C.; whereas in short-haired dogs, or in long-haired dogs which had been closely shorn, the critical temperature was nearer 31°C.

With more precise methods than those available to Rubner, Benedict and MacLeod (1929) have determined that the rat's critical temperature

lies essentially between 28°C. and 32°C. We can find no reasonably recent studies of the critical temperature of the adult rabbit. Giaja (1925) states that the newly-born rabbit's critical temperature is 34°C.; Draize and Tatum (1932) used 33°C. for their metabolic studies with the adult rabbit. Neither of these sources gives original data to substantiate this point. As a matter of fact, a careful appraisal of the literature suggests that an animal's critical temperature cannot be regarded as a fixed thermal point. The critical temperature seems to lie within a limited range of several degrees centigrade, because the rate of heat production does not change appreciably within such limits.

This survey indicates that the critical temperature of the rabbit (a long-haired animal) lies reasonably close to 28°C. Our observations show that the choice of this temperature is satisfactory, because our metabolic rates (average of 2.18 cal. per kgm. per hour) are lower than the average of 2.62 calories per kilogram per hour which was reported by Haney and his collaborators (1930, 1932).

In addition to the effect of temperature upon the basal rate of metabolism, it is also known that the extent of the metabolic effects of thyroxine or anterior hypophyseal thyreotropic hormone depend in part upon the environmental temperature at which the animals are kept and at which their rate of metabolism is determined (Draize, 1930; Riddle et al., 1936; Collip and Billingsley, 1936). It is well established that a marked increase in metabolism results from such therapy when the environmental temperature is within the range of the animal's critical temperature; if the environmental temperature is at or below room temperature, an actual decrease in metabolic rate may occur from the same dose of these hormones. The environmental temperatures at which Haney made his observations would obviously have been unfavorable to the occurrence of the increases in metabolism which he reported.

Because of our lack of success with Haney's original technique we modified this method in several important respects. By the use of Bradford Cannon's electrodes, the stimulation of sympathetic fibers in the present experiments was accomplished with greater ease and less trauma to the nerve than was possible by Haney's technique. The latter consisted of cutting the sympathetic trunks low in the neck, tying a thread to the free end of the cephalic segment, and alternately raising and lowering the nerve fibers by means of the thread in order to bring them in contact with the unshielded electrodes as he shifted stimulation alternately from one side to the other.

We considered the rate at which the autonomic fibers were stimulated as an additional factor of importance in this type of experiment. Haney used brief volleys of tetanic shocks to stimulate the cervical sympathetics in his experiments. Having tried his technique and failed to repro-

duce his results, we used what has been shown to be a physiologically more effective rate of stimulation for autonomic nerve fibers, viz., 20 to 30 per second (Bishop and Heinbecker, 1932; Knoefel and Davis, 1933; Orías, 1932; Rosenblueth, 1932). This technique was likewise unsuccessful.

One of the most important points which one may question in Haney's technique is his method of determining the heat production. He calculated the metabolic rate of his rabbits from the weight of the CO_2 exhaled per unit of body weight and time. Reference to Carpenter's tables (1921) discloses the fact that an error of 32.6 per cent can be made in the calculation of the basal metabolism from the calorific value of CO_2 if the R.Q. shifts from 0.7 to 1.0; whereas, a variation of only 7.7 per cent occurs in the calculation of the metabolism from the calorific value of oxygen if a similar shift in R.Q. takes place. It is quite unlikely, judging from the gastro-intestinal physiology of the rabbit, that the R.Q. remains constant from day to day, even after the 18- to 24-hour fast which must precede the determination of its metabolic rate. Marine and Lenhart (1920) and Marine and Baumann (1921) have shown that the R.Q. may vary the entire possible range in individual rabbits which are observed over a period of days under what are designated as standard conditions.

In addition to the points which have been raised already in connection with Haney's experiments (1932), one may also question the interpretation of some of his data, viz., that the thyroid hormone is partly liberated at the time of nerve stimulation. He bases this inference on the observation that the metabolic rate of animals thyroidectomized immediately after nerve stimulation is distinctly higher during the first 48 hours than the postoperative metabolism of rabbits thyroidectomized but not stimulated (Haney's tables 3a and 3b). But comparison of these data with those in another part of his paper (Haney's tables 2a and 2b) show (1) that these differences are within the limits of his experimental error; and (2) that these post-operative increases in metabolism (Haney's table 3a) were actually *smaller* than those noted after cervical sympathetic stimulation in completely myxedematous rabbits (Haney's table 2a). Haney himself finds that during the first 48 hours after cervical sympathetic stimulation the rate of oxygen consumption of myxedematous rabbits increased as much as that of rabbits with intact thyroids. In both groups of animals the metabolic rate increased an average of approximately 18 per cent above normal. One cannot determine from the unpublished data what the individual increases were in the group of myxedematous rabbits, but it is possible that some of them substantially exceeded 18 per cent. These comments are based on Haney's data labelled: "average percentage variation from individual normal" which represent conditions as they were found in individual rabbits. Such data are obviously more reliable than those based on an average normal for the general group of rabbits.

Finally, it is contrary to what is known about the thyroid gland to postulate that a single period of cervical sympathetic stimulation, limited to several hours, can induce physiological changes which would accelerate its secretion of a metabolism-stimulating hormone for a period of 4 to 6 weeks. The most powerful stimulant to the thyroid gland is acknowledged to be the thyreotropic hormone of the anterior hypophysis. An adequate single dose of this hormone raises the metabolism of a sensitive animal such as the guinea pig for only a few days; even similar daily injections for 4 to 6 days fail to stimulate the gland beyond the point where injections are discontinued. As Haney suggests, the prolonged rise in metabolism cannot be due to any thyroid hormone which is presumably liberated at the time of stimulation. He assumes, therefore, that stimulation of the sympathetic nerve fibers sets in motion a mechanism which maintains the increased secretory rate of the gland for several weeks after stimulation is discontinued. Such a phenomenon has not been described for any other gland of internal or external secretion.

The results of our experiments, therefore, are not in accord with Haney's; and the indisputable proof of a functionally active innervation for the thyroid gland is still lacking. Nevertheless, one is loath to reject completely the possibility that the nervous system can affect the functional activity of the thyroid gland. Perhaps the relationship is more subtle than the one for which we have been searching.

SUMMARY

The cervical sympathetic nerves of 22 rabbits were stimulated faradically for periods of one-half to four and a fourth hours at varying rates of stimulation. In 12 of these (group A) there was no significant change in the basal rate of oxygen consumption. The basal metabolism did increase to some extent in 8 rabbits (group B), but these increases fluctuated irregularly and were not maintained at an elevated level for more than a few days. The basal metabolism increased considerably for several days in the remaining 2 rabbits (group C). In one of them, autopsied and its thyroids examined at the height of the increase in metabolism, the gland exhibited none of the characteristic histological signs of hypersecretion. In view of this observation, there is no obvious explanation for the increased metabolism in this case or in the animals of group B. Vagal stimulation did not change the rate of metabolism in 2 rabbits (group D).

These results do not support the view that the rate of secretion of thyroglobulin is accelerated by excitation of the cervical sympathetic nerves alone.

We wish to express our appreciation to Dr. W. B. Cannon, who suggested this investigation, for his guidance and advice.

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ELECTROCARDIOGRAPHIC CHANGES AND CONCENTRATION OF CALCIUM IN SERUM FOLLOWING INTRAVENOUS INJECTION OF CALCIUM CHLORIDE[1]

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Electrocardiographic changes during or following the intravenous injection of calcium salts have been observed by several investigators (1, 3, 5, 6, 8, 12, 14). The results have not been entirely consistent, as might indeed be expected both from the fact that different animal species were studied, and from the variability in the amount of calcium injected, the concentration of the solution, and the duration of the injection. Comparison and interpretation of the changes recorded in these reports are difficult, since in none was the concentration of calcium in the serum during the course of injection determined. The present study is a reinvestigation in the intact animal of the effects of greatly varying concentrations of calcium in the blood. To this end calcium chloride solution was injected at a uniform rate sufficient to produce a continuously rising concentration of calcium in the serum, and at intervals during the injection blood samples were withdrawn and analyzed for calcium. It was thus possible to follow in sequence the appearance and evolution of the various electrocardiographic changes, and to compare them with the simultaneous concentrations of calcium in the serum.

PROCEDURE. The general procedure was that described in a previous communication (13). Twenty adult dogs were used. The animals received morphine sulfate subcutaneously, 10 mgm. per kgm. body weight, 30 minutes before the injection of calcium salt was begun. Four animals received sodium amytal 50 mgm. per kgm., instead of morphine sulfate. Six dogs, in addition to the morphine sulfate, received atropine sulfate, 0.2 mgm. per kgm., intravenously 5 minutes before receiving the calcium. Calcium chloride solution (0.205 M) was injected through a cannula in the femoral vein at rates varying from 0.2 to 7.4 cc. per kgm. per min. Samples of blood were withdrawn at various intervals, and the concentration of calcium in the serum measured by the method of Kramer and Tisdall (7). The concentration of inorganic phosphate in the serum was determined in each sample by the method

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of Fiske and Subbarow (4). Blood specimens were taken from the jugular vein except the sample at the moment of death, which was obtained by direct cardiac puncture. Electrocardiograms from lead II were taken at frequent intervals during the course of the injection.

RESULTS. Protocols of all experiments are summarized in table 1. The experiments are grouped according to the nature of the previous medication, and within each group they are arranged in ascending order according to the rate of calcium injection. The concentrations of calcium were plotted against time, and the concentrations corresponding to the different electrocardiographic changes were obtained by interpolation. These interpolated values appear in table 2. The electrocardiographic changes will first be described; their association with changes in the calcium and phosphorus concentrations of serum will be considered separately.

Sequence of electrocardiographic changes. Calcium in animals with only a preliminary injection of morphine. Cardiac inhibition was one of the earliest and most constant manifestations associated with an increase in the concentration of calcium in the serum, appearing in eight out of ten experiments (fig. 1). Usually both slowing and auriculo-ventricular block of all degrees were found, although occasionally pronounced slowing with no block occurred, and once total block with a normal auricular rate was maintained for several minutes. Occasionally slowing was accompanied by the appearance of nodal escape at an interval somewhat greater than that between the preceding normal beats. In one experiment temporary auricular fibrillation was found at this stage.

Succeeding the preliminary phase of inhibition, or partially superimposed upon it, there appeared, in every instance, a phase of enhanced automaticity. Most simply this phase appeared as an acceleration of cardiac rate, replacing the previous slow rate. More frequently the slow basic rhythm was interrupted by isolated extrasystoles at rather long intervals (0.6–1.2 sec.) or by coupled extrasystoles at short intervals (0.20–0.30 sec.) (fig. 2D). Either spontaneously or following such coupled extrasystoles, there appeared tachycardias from single or from multiple foci. In 5 of the 10 experiments with morphine alone, this phase of increased automaticity terminated in ventricular fibrillation (fig. 2E). In 2 experiments the sudden appearance of ventricular fibrillation was itself the first evidence of the onset of the rapid phase.

The 5 animals surviving the period of increased automaticity succumbed eventually to cardiac arrest. The phase of acceleration began to be replaced by a second period of inhibition. The rate of ventricular tachycardia became slower, the escapes less numerous and at a slower rate, and short periods of arrest were interpolated in the rhythm (Luciani periods). Normal complexes often returned. The periods of arrest, at first only 2 to 4 seconds in duration, increased to 8 or 10 seconds, and

TABLE 1

Summary of experiments

EXPERIMENT NUMBER	WEIGHT DOG	DRUGS INJECTED	CALCIUM CHLORIDE INJECTED (0.205M SOLUTION)		TIME FROM START OF INJECTION	CONCENTRATION IN THE SERUM		MODE OF DEATH
			Amount	Rate per min.		Ca	P	
	kgm.		*cc.*	*cc. per kgm.*	*minutes*	*mgm. per cent*	*mgm. per cent*	
1	12.2	Morphine	99	0.5	0	11.2	5.4	Fibrillation
					18	51.4	3.8	
2	9.1	Morphine	280	0.7	0	10.2	5.4	*
					6	23.4	4.8	
					15	39.0	5.2	
					28	44.4	4.7	
					46	45.6	5.0	
3	10.5	Morphine	66	0.8	0	10.6	3.3	Fibrillation
					8	53.0	2.5	
4	6.8	Morphine	149	0.8	0	10.7	4.3	Arrest
					5	41.4	3.6	
					14	65.4	3.1	
					26	106.4	1.4	
5	12.6	Morphine	89†	1.0†	0	10.0	3.5	Fibrillation
					7	56.8	2.7	
6	7.5	Morphine	113	1.1	0	10.9	4.1	Arrest
					14	70.8	3.7	
7	7.5	Morphine	142	1.1	0	10.0	2.9	Fibrillation
					14	49.4	3.3	
					18	60.0	3.0	
8	8.8	Morphine	130‡	1.5‡	0	10.3	4.2	Arrest
					7	85.9	2.0	
					10	163.7	0.7	
9	8.8	Morphine	209	2.6	0	11.5	5.8	*
					9	123.5	1.2	
Injection stopped at end of 9 min. Heart temporarily stopped by arrest								
					34	63.4	3.5	
					56	52.4	4.8	
10	11.6	Morphine	86	7.4	0	10.3	2.6	Fibrillation
					1	95.4	5.5	
11	9.0	Amytal	175	0.7	0	10.9	6.1	Fibrillation
					17	51.8	2.8	
					27	90.6	1.5	
12	6.1	Amytal	103	1.5	0	10.7	7.0	Arrest
					11	189.2	0.9	
13	10.7	Amytal	278	1.6	0	10.7	6.2	Arrest
					6	37.4	5.7	
					16	91.2	1.7	
14	18.2	Amytal	304	1.7	0	10.7	5.9	Arrest
					6	85.6	1.8	
					10	111.4	1.4	

TABLE 1—*Concluded*

EXPERIMENT NUMBER	WEIGHT DOG	DRUGS INJECTED	CALCIUM CHLORIDE INJECTED (0.205M SOLUTION)		TIME FROM START OF INJECTION	CONCENTRATION IN THE SERUM		MODE OF DEATH
			Amount	Rate per min.		Ca	P	
	kgm.		*cc.*	*cc. per kgm.*	*minutes*	*mgm. per cent*	*mgm. per cent*	
15	14.2	Morphine	31	0.2	0	10.5	4.0	Fibrillation
		Atropine			12	31.3	4.8	
16	6.0	Morphine	33	0.3	0	9.7	4.5	Fibrillation
		Atropine			9	36.8	5.5	
					22	44.8	5.5	
17	11.7	Morphine	210	0.7	0	10.2	5.4	Arrest
		Atropine			8	75.4	4.9	
					26	82.4	1.5	
18	4.0	Morphine	36	0.8	0	11.0	2.5	Fibrillation
		Atropine			11	70.4	1.6	
19	10.2	Morphine	162	1.5	0	10.5	5.8	Arrest
		Atropine			5	51.8	4.0	
					11	85.4	2.4	
20	9.3	Morphine	41	1.5	0	10.8	4.0	Fibrillation
		Atropine			3	51.2	2.8	

* Injection was stopped before death in these 2 animals. Both died some hours later.

† 0.308 M $CaCl_2$ injected; calculated in terms of 0.205M.

‡ 0.103 Ca lactate injected; calculated in terms of 0.205M.

the sequences of beats between these periods of arrest came less frequently and were themselves slower in rate. In the final stage complete stoppage for 0.5 to 2 minutes occurred, interrupted only by a single or a short series of ventricular beats, and lastly final and total arrest supervened (fig. 1). The point of final complete arrest was somewhat indeterminate since isolated beats might occur after long periods of complete arrest. This uncertainty is emphasized by the fact that attempts at this point to revive the animals by artificial respiration and cardiac compression were temporarily successful in two instances. The introduction of the needle to withdraw a cardiac blood sample after arrest occasionally induced ventricular fibrillation. This occurred only as a post mortem reaction.

Even before any alterations in rate or rhythm appeared, typical changes in the contour of the ventricular complex were noted. These were in every way similar to those found in the rabbit by Hoff and Nahum (6) and consisted of 1, a widening of the Q R S complex; 2, disappearance of the S-T interval; 3, displacement of the S-T take-off, and 4, characteristic changes in the T wave. They were greatly obscured in these experiments by the appearance of such a variety of ectopic beats, and are shown

better in later experiments in which these arrhythmias were in great measure suppressed.

A feature not noticed by Hoff and Nahum in the rabbit was the appearance in 3 experiments of a secondary summit after T which is in all probability a U wave (fig. 1).

Calcium in animals anesthetized with amytal. Injection of calcium in animals previously anesthetized with sodium amytal had qualitatively the same effect as in animals given morphine, both in its influence on

TABLE 2

Concentration of calcium in milligrams per cent at which the more important electrocardiographic changes appeared

EXPERIMENT NUMBER	T-WAVE CHANGES	FIRST SLOWING PHASE	A-V CONDUCTION CHANGES	FIRST RAPID PHASE	VENTRICULAR FIBRILLATION	SECOND SLOWING PHASE	ARREST WITHOUT FIBRILLATION
1	13	13	13	35	51		
3	50	38	—	50	53		
5	12	22	22	—	57		
7	20	—	—	40	60		
10	50	—	—	—	95		
11	—	30	—	—	91		
15	30	—	—	—	31		
16	—	13	22	—	45		
18	16	52	—	—	70		
20	40	—	—	—	51		
4	—	18	18	57		83	102
6	—	18	—	60		—	71
8	—	30	—	60		105	164
12	25	—	65	90		—	189
13	12	—	—	55		83	91
14	20	—	40	60		86	111
17	22	—	—	—		—	82
19	28	—	—	23		50	85
2	—	23	41	41		45	—
9	20	—	—	35		—	124
Averages.....	25	28	33	48	60	92	113

rate and rhythm and in its effect on the ventricular complex. However, both the earlier stage of inhibition and the succeeding period of acceleration, though present, were less marked. The preliminary inhibition was limited in 3 of the 4 experiments to a progressive prolongation of the P-R interval which did not reach the stage of partial block, with no clearly recognizable change in rate. The period of acceleration was slight in degree and duration. Arrest occurred while the rhythm was entirely normal. In the fourth experiment slowing to almost half the control rate took place, and shortly after the appearance of a few coupled extrasystoles,

ventricular fibrillation developed. Mechanical stimulation of the hearts in which arrest took place evoked ventricular fibrillation in two.

These experiments, because of the almost complete absence of ectopic beats, serve to portray clearly the progressive changes in the Q R S and T complexes mentioned above. They also demonstrate the effect of calcium on the U wave.

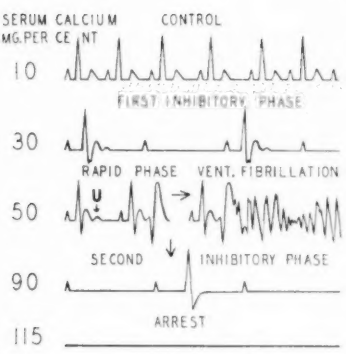

Fig. 1

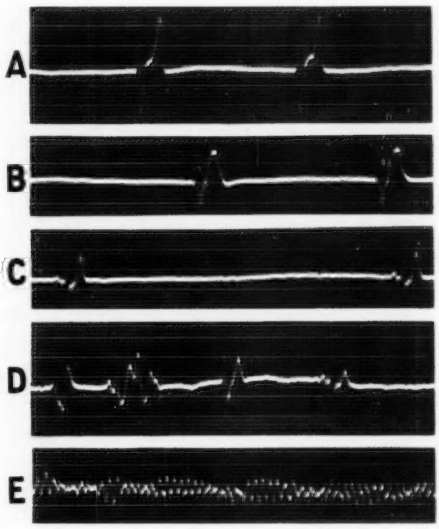

Fig. 2

Fig. 1. Diagram indicating the principal electrocardiographic changes produced by elevation of serum calcium. The electrocardiograms show both the alterations in rate and rhythm, and the changes in the ventricular complex. At the left are given in round numbers the average concentrations of serum calcium at which the several electrocardiographic changes occur.

Fig. 2. Experiment 3. A. Control. B (Ca 22 mgm. per cent), and C (Ca 38 mgm. per cent). Slowing phase showing disappearance of ectopic rhythm. D (Ca 49 mgm. per cent). Onset of rapid phase with a ventricular extrasystole on the descending limb of a typical calcium T wave. E (Ca 53 mgm. per cent). Sudden onset of ventricular fibrillation.

Calcium in animals receiving atropine in addition to morphine. With only minor exceptions the early bradycardia was entirely suppressed by atropine. At the same time the phase of acceleration was greatly enhanced in degree. Ventricular extrasystoles were more frequent, came earlier in the cycle, and culminated in ventricular fibrillation in 4 of the 6 experiments. In the other 2 experiments arrest appeared suddenly in a heart which was beating at a rate equal to or greater than normal. In these

experiments also the typical changes in the ventricular complex are clearly portrayed.

Correlation of electrocardiographic changes with the concentrations in the serum of calcium and of inorganic phosphate. The concentrations of calcium at which the various electrocardiographic changes first appeared are presented in table 2. It appears at once that there is a considerable variation in the concentration of calcium in the serum at which each of the major changes occurs. Critical levels of calcium at which each manifestation appears do not exist, but ranges of concentration are clearly correlated with each of the electrocardiographic changes. The first rapid phase and ventricular fibrillation appear regularly at a significantly higher level of calcium than the T wave changes and the first inhibitory phase; while final arrest without fibrillation appears only at much higher levels than those at which fibrillation develops.

The inorganic phosphate of the serum (table 1) was usually lower during the calcium injection, but the exact level was irregular and unpredictable from the calcium concentration. No relation of phosphate changes to the other variables is apparent.

In an attempt to determine the mode of combination of the excess calcium in the serum an ultrafiltrate was prepared of the third serum sample of experiment 2. The calcium in the ultrafiltrate was 156 mgm. per cent compared with 163 mgm. per cent in the original serum, indicating that most of it was in a freely diffusible form.

Mode of death and fatal dose. The final column of table 1 indicates whether sudden ventricular fibrillation or gradual arrest caused death. The total volume of calcium solution given (column 4 in table 1) represents the amount necessary to produce death under the conditions of the experiment. The amounts given varied greatly from animal to animal. Significantly greater amounts were needed to produce death in animals by arrest than by fibrillation, corresponding to the definitely higher levels of the calcium of the serum at which arrest occurred. Otherwise the fatal dose can not be correlated with rate of injection, type of preliminary medication, or final serum concentration of calcium; the striking fact is the variation from animal to animal. This is in accord with the observations of Lieberman (9).

Potassium was found to cause fibrillation more frequently when injected rapidly than when injected slowly (11). The rate of injection of calcium has no such influence. Fibrillation and arrest both occurred whatever the rate of injection.

All animals were autopsied immediately and it was invariably found that the hearts of those dying of fibrillation were widely dilated, and so gorged with blood that the pericardium was under considerable tension. Hearts in arrest without fibrillation were neither in extreme systole nor

diastole, but mid-way between, and responded to mechanical stimulation by vigorous and effective contractions. These latter hearts not infrequently were sent into ventricular fibrillation by the manipulation at autopsy, or more often by the introduction of the large gauge needle to withdraw blood for the final sample. That death was truly of circulatory origin is indicated by the fact that respiration continued for several minutes after cardiac failure from arrest or ventricular fibrillation.

Arrest occurred in 3 out of the 4 experiments in which amytal was given. This is a higher proportion of deaths by arrest than in the morphine group, but the series is too small to eliminate the possibility that this difference was a chance effect. Similarly no difference in the final mode of death appeared between the atropinized animals and the others. The low incidence of extrasystoles with amytal, and the increased frequency with which they appeared with atropine suggested, however, that these drugs may influence the mode of death in dogs as they do in rabbits (Hoff and Nahum, 6).

A third toxic action of calcium is probably indicated by the death of two animals an hour or more after they had seemingly recovered from the immediate effects (cardiac arrest) associated with a high serum calcium. In one of these animals (expt. 9) the concentration of calcium in the blood sample taken an hour after discontinuing calcium administration was still almost five times the normal value (table 1).

Discussion. The experiments reported here serve to explain in part the divergent reports of cardiac changes resulting from the intravenous injection of calcium chloride. It has been seen that the action of calcium on the heart appears in three different phases: 1, a preliminary inhibitory phase; 2, a subsequent period of acceleration, sometimes terminating in ventricular fibrillation; and 3, a final stage of slowing and arrest, occurring in all those animals which escaped ventricular fibrillation during the second phase. Although each of these phases varied widely in character and although the different phases tended to overlap somewhat, the division into these three major phases is an entirely natural one.

Atropine entirely abolished the first inhibitory phase. This alone strongly suggests that this inhibition is due to vagus stimulation. Such an interpretation is supported by the fact that death never occurred during this first phase. The interpretation of the rapid phase is less clear. It is, if anything, intensified by atropine, which indicates that it can not be due to parasympathetic activity. From these experiments it can not be decided whether the increased cardiac activity results from sympathetic stimulation or a direct effect on the myocardium.

Ventricular fibrillation was the only mode of death recognized by Walters and Bowler (12). In roughly half of our experiments, however, ventricular fibrillation did not occur. The phase of increased irritability

was succeeded by a phase of progressive depression of cardiac activity culminating in death by arrest; this has been termed the "third phase." This phase can be interpreted only as a direct depressant effect of calcium on the heart muscle.

A study of the serum calcium levels at which the various changes occur presents several points of interest. There are no exact critical levels at which the changes occur, which is quite different from the reaction to potassium (13). However, there is a distinct general tendency for specific changes to appear only after the calcium in the serum has reached a certain height; thus, the first rapid phase appears only once at levels below 30 mgm. per cent; the second slowing phase never below 45 mgm. per cent, and direct arrest never below 71 mgm. per cent. These figures are important because they indicate approximately what may be expected from intravenous injection of definite amounts of calcium chloride in animals and man. Thus, initial bradycardia and T wave changes appear at very low levels, easily reached temporarily by the injection of a few cubic centimeters of 10 per cent solution of calcium chloride. The levels at which tachycardia and ventricular fibrillation appeared are distinctly higher, but might, in exceptional instances, momentarily be reached by a rapid intravenous injection of a concentrated solution (ventricular fibrillation occurred at a level of only 31 mgm. per cent in expt. 15). Direct depressant effects appeared at definitely higher levels, in which the serum calcium ran to seven times normal or more, and could be reached only by the continuous intravenous administration of large amounts of calcium chloride

These considerations have a bearing on the toxicity of calcium chloride in man. There is one report of cardiac arrest in man (with subsequent complete recovery) following the intravenous administration of 4 cc. of a 10 per cent solution of calcium chloride (10). Judging from our experiments, this arrest could hardly have been due to a direct effect on the heart muscle (second slowing phase), since so little calcium was given. Rather it must have represented an unusual intensification of the first slowing phase, which we have tentatively identified as a vagus effect. There are reports (2, 14) of instant death in 3 cardiac patients following intravenous injections of 10 cc. of 10 per cent calcium chloride. These were almost certainly caused by ventricular fibrillation, since efforts at resuscitation had no success; this would be unusual were the cause simple arrest due to excessive vagus stimulation. Such an interpretation is consistent with our findings. The injection of 10 cc. of 10 per cent calcium chloride may conceivably produce a temporary concentration of calcium in the fluid surrounding the heart not far from the lower levels at which fibrillation has been shown to occur. The presence of antecedent cardiac disease in these cases may well have rendered the hearts more liable to develop

fibrillation. That such accidents must be rare is indicated by the frequency with which calcium chloride has been given intravenously in a great variety of conditions (12, 14), and the rarity of the reports of sudden death. But if the human heart behaves as does that of the dog, sudden ventricular fibrillation during calcium injection remains a possible, if unusual, accident.

SUMMARY

1. Dilute solutions of calcium chloride were injected intravenously into dogs at a uniform rate so that the concentration of calcium in the serum rose gradually and continuously. Frequent electrocardiograms were taken and determinations of calcium concentration in the serum were made at intervals until death; these were then compared.

2. A sequence of electrocardiographic changes appeared as follows:

a. First slowing phase; frequently associated with T wave changes and changes in the A-V conduction; apparent at calcium concentrations ranging from 15 to 65 mgm.

b. Rapid phase; increased automaticity frequently ending in ventricular fibrillation; apparent at calcium concentrations from 25 to 90 mgm. per cent.

c. Second slowing phase; general depression ending in cardiac arrest without fibrillation; occurring only in animals surviving the rapid phase; apparent at calcium concentrations of 70 to 190 mgm. per cent.

3. Death during acute experiments may be caused either by sudden ventricular fibrillation or by gradual cardiac arrest. Animals surviving the acute experiment may succumb later for some unassigned cause.

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MANNER OF STRYCHNINE ACTION ON NERVOUS SYSTEM[1]

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Strychnine action in the intact animal is characterized not only by a lowering of the threshold for reflex excitation of muscle contractions but also by an alteration in their intensity and duration. One theory (Bremer, 1925) is that strychnine action occurs entirely from lowered threshold at the nerve synapse. The possibility that accommodation might also be a factor in accounting for the results of strychnine action on the central nervous system, is suggested by information that has been accumulating from accommodation studies on the nerve axon. Realizing the difficulties of direct investigation of accommodation in the central nervous system it was deemed advisable to approach the subject by first investigating the effect of strychnine on the properties of the axon, on the fibre groups, and on the peripheral synapse. Certain studies on the effect of strychnine, on the peripheral axon (Peugnet and Coppee, 1936), and on isolated ganglion cells or ganglionic cell groups were already available (Heinbecker, 1932). With such data in hand, experiments were planned for the spinal cord and brain, designed to yield information capable of comparison with the results of the studies on peripheral structures, it being assumed that, in ultimate analysis, the action of strychnine on comparable structures would be similar throughout the nervous system. This report contains the data on which such a comparison was made and a discussion of the physiological implications resulting therefrom.

METHODS AND RESULTS. *The effect of strychnine on the form of the axon potential.* The effect of strychnine on the form of the axon potential was investigated in the alpha group and also in single fibers. In one series of five experiments potentials just above threshold for the alpha fibers and of constant amplitude, as long as this was possible without increasing the stimulus strength more than 400 per cent above its initial value for each nerve, were recorded from frog sciatic nerves at the point of stimulation. The stimulating electrodes were connected into one arm of a Wheatstone

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bridge to eliminate large escapes of current into the leads. The potentials were made monophasic by crushing under the grid electrode and applying 2 per cent cocaine. Strychnine in concentrations of 1 to 1,000,000; 1 to 200,000; 1 to 100,000; and 1 to 1,000 was applied to the region of the vertically placed stimulating electrodes by a drip method. After definite depression of the potential was evident, washing with Ringer's solution was carried out and the effect on the potential again observed.

For all concentrations studied the effect of strychnine was to gradually lower the potential of the alpha fibers with no increase in duration and with a gradual decrease in area.

In another series of fifteen experiments the effect of strychnine on single axon responses, made monophasic by crushing and cocainizing under the grid electrode was observed in frog nerve. The preparations extended from the root region down to the distal part of the tibial nerve. The stimulating electrodes were applied to the root and the recording electrodes

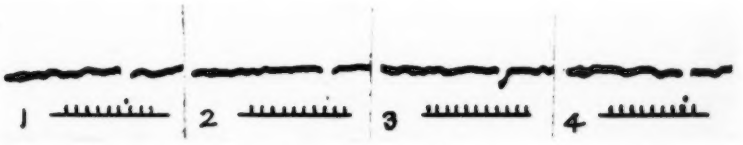

Fig. 1. Records of single axon potentials. *1*, before the application of strychnine. *2*, 5 minutes after application of 1:200,000 strychnine. *3*, 15 minutes after application of strychnine. *4*, after 10 minutes' subsequent washing with Ringer's solution. Time line below responses, designates milliseconds. Note that there is no essential change in duration from strychninization and a gradual diminution in area. On washing with Ringer's solution recovery to normal takes place.

to the fine distal part of the nerve. Strychnine in concentrations varying from 1 to 1,000,000 to 1 to 1,000 was applied to the region of the stimulating electrodes. After definite depression of the potential was evident washing with Ringer's solution was carried out to remove the strychnine and the effect on the potential again observed.

Strychnine (1 in 1,000,000 and less) depresses individual axon spikes of the green frog sciatic without change in their duration other than that due to internodal delay. There is a gradual decrease in area (fig. 1). Only A fibers were so investigated, but all our previous studies would indicate that the other fibers would be similarly affected. We are unable to account for the difference in our experimental findings and those of Peugnet and Coppee who found evidence of an increase in area and a marked prolongation of the fiber potential.

Effect of strychnine on fiber groups. The effect of strychnine on nerve axon groups was investigated in both the rabbit's and the cat's saphenous and vagus nerves. The saphenous fiber groups are so dispersed as to

yield recognizable A, B, and C potentials (Heinbecker, O'Leary and Bishop, 1933) while the vagus potential after some conduction exhibits A, B_1, B_2, and C waves (Heinbecker and O'Leary, 1933). Strychnine sulphate in varying concentrations from 1 in 10,000,000 to 1 in 100 in Tyrode's solution was applied by the drop method to the region of the stimulating or recording electrodes as the occasion required. Condenser discharges served as stimuli.

In experiments designed to determine the order of depression in the fiber groups, it was found that with concentrations of strychnine varying from 1 in 10,000 to 1 in 100, in both the cat and the rabbit, the small somatic myelinated fibers, responsible for the B_1 wave, are first depressed to extinction. The B_2 or autonomic myelinated fibers are next, the A and C groups last and at approximately the same rates. The findings agree with those of Peugnet and Coppee (1936, loc.cit.). With a concentration of 1 in 500, the time required for extinction of A and C waves averages 15 to 30 minutes when the strychninized Tyrode's solution is allowed to drop over the nerve trunks at the rate of 20 to 30 drops per minute.

Effect of strychnine on C fiber response. To study the effect of strychnine on the spike and on the negative and positive after-potentials of the C fiber, cat and rabbit vagus nerves were used. The grid and ground recording electrodes were widely separated (6–10 cm.). The nerve under the grid electrode was crushed and treated with 2 per cent cocaine. The result was a monophasic record in which the C potential is followed by a a negative and positive after-potential (Bishop, 1934). The effect of applying strychnine (1–10,000) in the region of the ground electrode was observed.

In five experiments the only observable effect with our concentration of strychnine was a symmetrical lowering of the spike and both phases of the recorded after-potentials, presumably indicating that the positive and negative phases are both depressed and at about the same rate. Since it is uncertain to what extent the two phases of the after-potential overlap, it is difficult to be absolute about the effect of the drug on either phase. This finding would seem, however, to cast doubt on hypotheses in which long potentials associated with repetitive fiber responses in the central nervous system are considered to be fiber after-potentials from the region of their synaptic endings, since there, under degrees of strychninization which depress after-potentials in fibers, the long cortical potentials are still greatly increased.

Recovery of C fiber from strychnine poisoning by washing with Tyrode's solution was found to be extremely slow. In concentrations higher than 1 in 10,000 it generally did not occur.

Effect of strychnine on electrical threshold, absolutely refractory period, and conduction rate of fibers. In the excised nerves of the cat

and rabbit the threshold is invariably raised by any concentration of strychnine over 1 to 1,000,000. In lower concentrations, the results were variable. Sometimes moderate lowering (5–10 per cent) of the threshold is seen, but in general there is no change or a slight raising (10–20 per cent). An analysis of the results of all threshold experiments leads to the conclusion that warm blooded nerves studied *in vitro* do not show as much lowering of threshold with low concentrations of strychnine as was found by Peugnet and Coppee (1936) for cold blooded nerves. As with frog nerves, the concentration 1 in 1,000,000 seems to be a critical one above which depression invariably results, while for lower concentrations some degree of enchancement may result. The effect of strychnine on the threshold in frog nerve was studied with results identical with those reported by Peugnet and Coppee (1936, loc.cit.).

The effect of strychnine on absolutely refractory period of the alpha fibers for all concentrations used was to increase its duration. Conduction time likewise was always increased.

Effect of strychnine on accommodation in the nerve fiber. The effect of strychnine on accommodation was investigated in green frog sciatic and in rabbit vagus and tibial nerves (20 preparations). Only fresh nerves were used because it was found that preparations soaked in Ringer's solution for several hours sometimes failed to accommodate to cathodal polarization to a normal degree. Strychnine in concentrations varying from 1 to 1,000,000 to 1 to 50,000 was applied by the drip method to the vertically suspended nerve in the region of the stimulating electrodes. A cathodally directed polarizing current adjusted before each series of observations to be just subrheobasic in intensity and of 200 milli-seconds' duration was applied once a second to the nerve. A condenser shock (0.001 mfd. delivered through 35 to 40,000 ohms resistance) of a strength just adequate to yield a response 20 to 50 mm. in amplitude at the point of maximum effectiveness of the two stimuli, was superimposed through the same electrodes at various intervals during the flow of the polarizing current. Twenty-five thousand ohm resistances were inserted into the positive arms of the stimulating circuits to separate the galvanic from the condenser stimulus circuit. The height of the response to the shock stimulus at the various intervals throughout the flow of the polarizing current was marked on translucent paper placed over the face of the oscillograph. This permitted a series of readings to be made very rapidly, thus eliminating the effect of changes in the state of the nerve with time. Readings were made on both normal and strychninized nerves. After a definite change in accommodation was produced by strychnine, washing with Ringer's solution was begun and the degree of accommodation again determined.

In another series of five experiments the amplitude of the response to the shock stimulus was measured at various intervals after cessation of the

constant current in normal and strychninized preparations to study the recovery time.

The effect of strychnine on accommodation in the axon (fig. 2) is to inhibit markedly its development. The time rate of local potential change on application of the rectangular subrheobasic current was not altered

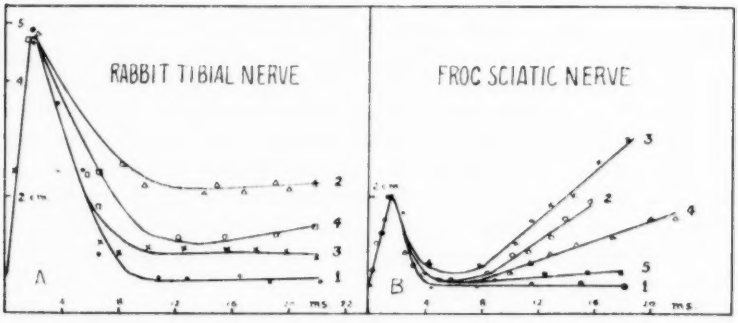

Fig. 2. Curves showing the relative amplitude of axon responses to faradic shocks superimposed upon a just sub-rheobasic cathodally polarizing current at various instants (abscissa) following its onset. The fall of the curves from their peaks is determined by the rate and amount of *accommodation*.

A. Curves of results of an experiment on a single tibial nerve of the rabbit. In all curves *R* indicates strength of the just subrheobasic current used; *V*, the voltage applied to the condenser (0.001 mfd.) for the shock stimulus. *1*, response height in millimeters at various instants after start of polarizing current for the nerve before use of strychnine. R 210 mv. V 4.5. *2*, response height after 10 minutes' washing with 1:20,000 strychnine. R 610 mv. V 6.0. *3*, response height after 12 minutes' washing with Tyrode's solution. R 500 mv. V 6.0. *4*, response height after a second 13 minutes' washing with strychnine. R 390 mv. V 9.0. Interelectrode distance 9 mm. Conduction distance 48 mm. Silver-silver chloride electrodes used. Similar results secured with non-polarizable calomel electrodes.

B. Curves of results of similar experiments on the sciatic nerve of frog. *1*, response height before application of strychnine. R 260 mv. V 9.0. *2*, response height after application for 10 minutes of 1:50,000 strychnine. *3*, response height after 21 minutes' washing with strychnine. R 440 mv. V 6.0. *4*, response height after 10 minutes' subsequent washing with Ringer's solution. R 420 mv. V 6.0. *5*, response height after washing in Ringer's solution for 10 minutes a second time. R 470 mv. V 6.0.

Note that strychnine diminishes the degree of accommodation. Washing with Ringer's solution causes a definite return to or toward normal indicating that the effect is not due to degeneration.

significantly by strychnine as evidenced by a lack of any appreciable change in the rising phases of the curves plotted in figure 2. It is of interest to note that on breaking the constant current the return of the threshold to normal for a condenser stimulus is much more rapid in strychninized nerve than in the normal. This result is to be expected on the basis of

theoretical considerations developed by Hill (1936) in his theory of excitation of nerve. Having found that strychnine inhibited the development of accommodation in nerve, it might be expected that repetitive responses would follow stimulation but under our experimental conditions they did not.

Effect of strychnine on the peripheral synapse. Strychnine action on a peripheral synapse was studied in the excised superior cervical ganglion of the turtle. This ganglion was chosen because it maintains a state of relatively normal function for a considerable time after excision. It has a preganglionic stretch of 20 to 30 mm. and a long post-ganglionic one of 40 to 60 mm. Concentrations of strychnine 1 in 10,000,000 to 1 in 10,000 in Tyrode's were applied by dripping the solution onto the ganglion. Condenser stimuli of small capacity (0.001 mfd.) were employed in order that the degree of overlap of a first and second stimulus required for the investigation of alterations in the period of latent addition might be as little as possible. It is realized that such an overlap is not completely avoided at the shorter time intervals under 1.5 to 2.5 ms. This must result in some error, making the period of latent addition appear longer than it really is but for basis of comparison between the normal and the strychninized preparation this method is considered satisfactory.

In six experiments it was found that the threshold of excitability of the synapse-neuron complex sometimes showed some lowering with concentrations less than 1 in 1,000,000. This was evidenced by an increase in area of the submaximal postganglionic response to the same strength of preganglionic stimulus. The increase was usually not more than 20 to 30 per cent and often did not show itself. At higher concentration depression was the invariable result. There was no evidence of an increase in the area of spike potentials recorded from the ganglion. The absolutely refractory period was invariably prolonged by the concentrations used.

Conduction through the ganglion is blocked in 15 to 30 minutes by a concentration of strychnine of 1 in 1,000. This is about the time required to block fibers of corresponding size in a nerve trunk. There was no evidence of any spontaneously aroused activity in the ganglion on strychninization and no repetitive responses ever followed single preganglionic stimuli. The cat's superior cervical ganglion in vivo was blocked in 15 to 30 minutes by 1 to 500 strychnine applied copiously about the ganglion.

The period of latent addition in the normal turtle ganglion was determined by applying to unequal brief condenser stimuli (the second always a little greater) submaximal for the first fiber group to the preganglionic trunk as indicated above and the stimulus separation determined at which the area of the first and second responses together just exceeded the area of the second response alone. It was found to be between 2 to 2.5 milliseconds (fig. 3). This is well within the refractory period of the fibers

concerned and shorter than the absolutely refractory period values found for the synapses (Heinbecker, 1930). The true period of latent addition must be within this value. Its actual value has not been determined because of the complication of stimulus overlap. After the application of strychnine the period of latent addition was lengthened 200 to 300 per

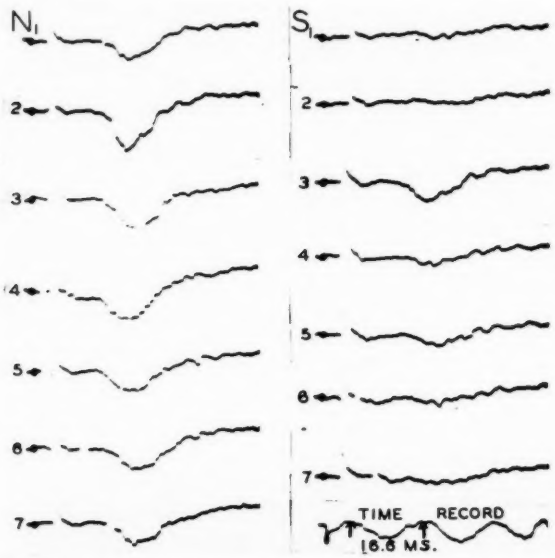

Fig. 3. Record to show increase in duration of period of latent addition after strychnine.

N 1. Record of response to second stimulus alone in normal nerve. Response of first stimulus alone (not shown) was about 50 per cent of this.

N 2, 3, 4, 5, 6, 7. Response to first plus second stimulus at varying intervals of separation between the stimuli. Note that the area of *A5* is just a little greater than that of *A1* above. Period of latent addition calculated to be 2.5 milliseconds.

S 1 and *2*. Record of response to second stimulus alone after strychnine. Response to first stimulus alone (not shown in record) was barely perceptible.

S 3, 4, 5, 6, 7. Response to first plus second stimulus at varying intervals of separation between stimuli. Note that in record 7 the response is still a trifle greater in area than that to the second stimulus alone. Period of latent addition calculated to be 6.5 milliseconds.

cent. This time is beyond any possible stimulus overlap. The lengthening still occurred even when the degree of blocking in the ganglion was marked and fatigue well developed.

Action of strychnine on isolated ganglion cells. The action of strychnine on individual ganglion cells or small groups of them has previously been studied in the median nerve cord of Limulus polyphemus (Heinbecker,

1936). The nerve cord was divided into small elements, some containing only a single ganglion cell of the large type. The record was thereby simplified, making the interpretation of drug and potential effects easier. These cells on the basis of the evidence presented at that time are regarded as pacemaker cells. Ganglion cells of this type singly or at least when one of them is associated with a group of small ganglion cells, themselves not spontaneously rhythmical, still exhibit rhythmical activity when isolated. From such preparations, spontaneous potentials of long duration and apparently simple, i.e., not the result of the addition of potentials of shorter duration, may be recorded. They are regarded as the expression of intrinsic activity in the large pacemaker ganglion cells because they are only found when these cells are present. On the rising and falling phases of the long potential, if of sufficient amplitude, short potentials are found superimposed, presumably representing the activity in the axons of the large pacemaker cells. The duration of the long potential in isolated preparations varies, depending partly upon the frequency of its appearance and the state of the preparation. With an increase in the duration of the long potential, the duration of appearance and often the amplitude and frequency of occurrence of the short potentials are increased. On the application of strychnine (1 to 2,000) the frequency, amplitude and total duration of the long potentials is increased, and with it an increase in duration of the period of short potential repetition and an increase in their frequency of discharge. With strong concentrations and a longer period of application all the potentials are depressed, finally to extinction. The amplitude and duration of the potentials are also modified by the extrinsic excitatory and inhibitory nerves. When the ganglion cell activity ceases as a result of the activity of the latter, all long potentials disappear which would probably not be the case if the cells were being inhibited by long potentials developed at synapses because at such times they should be maximal.

Effect of strychnine on the spinal cord. The action of strychnine on the spinal cord was observed in the turtle. The posterior third of the spinal cord was exposed from the dorsal side. An area 3 to 4 mm. long was delimited by induction shocks. In this region stimulation caused marked flexion of the hind limbs and just posterior to this a similar area for marked extension of the hind limbs. The sciatic nerve on one side was then stimulated centrally with graded condenser shocks applied at varying frequencies. The flexor and extensor muscles of the leg on the opposite side were cleared of skin sufficiently to permit the insertion of a pair of needle recording electrodes into one muscle typical of each group. Strychnine in varying concentrations was applied either diffusely or to finely localized areas on the dorsal and on the ventral side of the cord in these regions, and the effect on the crossed flexor and extensor reflexes studied. Similar

results were obtained with the circulation intact, without any circulation and also when the cord was severed above the levels being investigated.

On applying the strychnine to the dorsal cord only, the threshold for excitation of reflex effects was considerably lowered. When the strychnine was applied to the anterior cord only, the threshold for reflex effects was also definitely lowered. Most marked lowering of threshold occurred when strychnine was applied to the dorsal and ventral parts of the cord simultaneously.

It was noted when strychnine was applied to dorsal and ventral parts of the cord simultaneously that the application of a single shock adequate to simulate only a few of the alpha fibers resulted in a prolonged response from the anterior horn cells. This type of effect also followed to a lesser degree the application of strychnine to the anterior cord alone, but it was not seen on its application to the dorsal cord only. Repetitive stimulation was then still necessary to elicit the more prolonged type of response. This evidence suggests the possibility that in the turtle cord secondary neurons associated with dorsal root afferents on excitation may depolarize to a lesser degree than motor cells of the anterior horn when strychninized.

Summation within the cord of two subthreshold stimuli applied to the normal saphenous nerve occurred readily under strychninization. The summation was most effective when the shocks were together or within three to six milliseconds of each other. Responses would also occur at much longer intervals, 20 to 60 milliseconds, after several stimuli had been applied. This is interpreted to be the result of an alteration in the excitatory state of the synapse-neuron complexes involved.

Effect of strychnine on the brain. The effect of strychnine on the brain was studied in the cat and rabbit. Under nembutal or ether anesthesia the sensori-motor or optic cortex was exposed in one hemisphere and the opposite saphenous or optic nerve arranged for stimulation. The spontaneous cortical activity and its immediate response to stimulation were noted both before and after strychnine had been injected intraperitoneally or intravenously (1 to 3 cc. of 1 in 100 solution).

The effect of strychnine on the general cortical activity is shown in figure 4. There is an increase in area and frequency of the waves seen in the normal encephalogram. The excitatory effect may become great enough to produce a marked degree of synchronization such as was obtained by Adrian (1937) in his studies on the effect of strong light on the responses of an optic ganglion. The rate of such synchronized activity may reach 50 to 70 per second. A similar type of synchronization is often recorded from the sciatic nerves (fig. 4) after intravenous strychnine has aroused the anterior horn cells to activity. It may indicate the rate of axon response of the cells involved.

On stimulation of the saphenous nerve in the etherized animal, the first

response recorded from the cortex often reaches its maximum when not more than the first half of the alpha fibers are stimulated. Further excitation of other fibers throughout the nerve spectrum adds little to the amplitude and area of the distinguishable first response. After strychninization (fig. 5) there is a striking difference. The amplitude and area of the second and third waves of what has been termed the first response

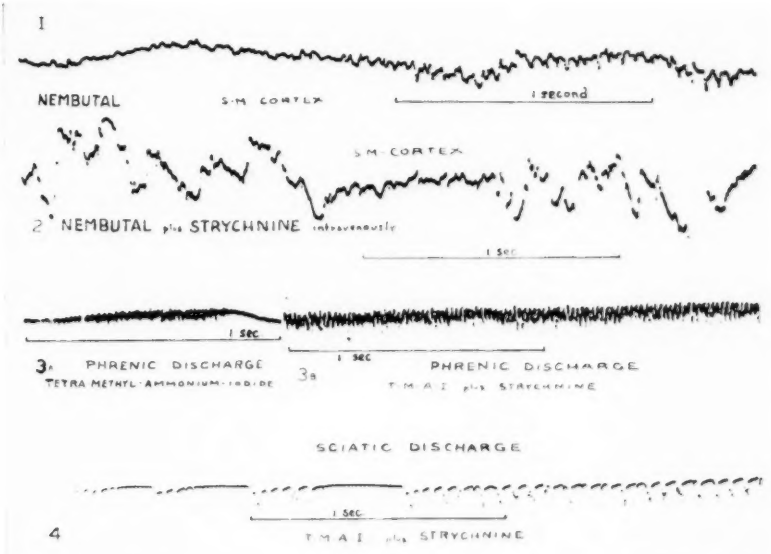

Fig. 4. 1. Record of spontaneous cortical activity under moderate nembutal anesthesia.

2. Record of spontaneous cortical activity after slow injection of 2 cc. of a 1 per cent strychnine solution diluted to 10 cc. in Tyrode's solution.

3. *a*. Record of normal phrenic nerve response under curarization with tetramethyl ammonium iodide.

b. Effect of intravenous strychnine on above.

4. Record from sciatic nerve of cat following intravenous strychnine on single stimulation of small fraction of saphenous alpha wave. Note that after 3 stimuli the activity becomes tetanic in nature. This activity persisted for some minutes.

(Bartley and Heinbecker, 1938) keep on increasing throughout the whole range of the fibers. The threshold for the first response is also somewhat lowered below the normal. It is most significant that the first wave of the "first" response increases much less in amplitude and duration than the second and third waves which are normally of much longer duration (fig. 6). This first wave is regarded as a pure fiber potential, the result of activity in the cortical afferents. It does not repeat following single

stimuli, but waves similar to the second and third waves may follow them in a manner suggesting that they are repetitive responses in the structures responsible for the second and third waves.

When paired submaximal stimuli, the first being weaker, are applied to the nerve at one second intervals, the cortical response to the first stimulus is unaffected by the second until the latter is brought to within 20 to 30 milliseconds of the former. From that point down toward zero

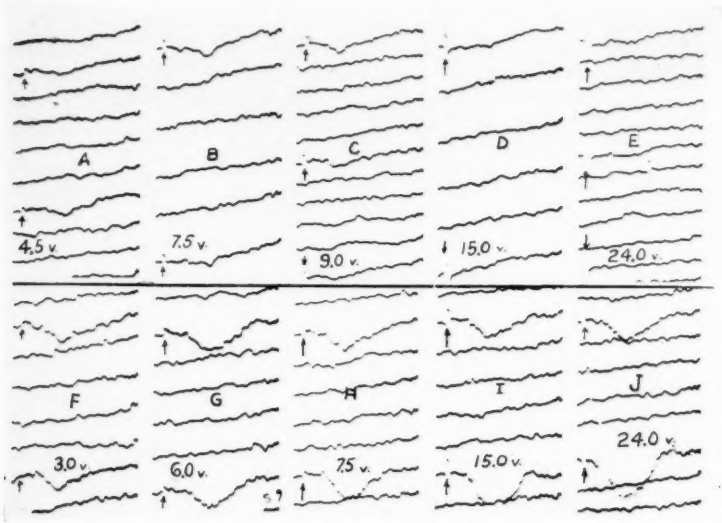

Fig. 5. Effect of strychnine on the first cortical response to saphenous nerve stimulation, ether anesthesia:

A, *B*, *C*, *D* and *E*: First response to progressively increasing stimuli, 4.5 to 24.0 volts, threshold for saphenous alpha wave 3 volts. Note that the area of the response does not change appreciably after 4.5 volts.

F, *G*, *G*, *I* and *J*: First response after strychninization by intravenous route to progressively increasing stimuli 4.5 to 24.0 volts, threshold for saphenous alpha wave 3 volts. Note that the amplitude and area of the total first response increases throughout the range of voltage applied.

separation time there is an increase in area of the first response. It is difficult to follow the changes when the two responses are very close together because of overlapping. When the brain is strychninized under similar circumstances, the second response adds to the first from 60 to 100 milliseconds inward towards zero separation time. This occurs when the threshold for the first response is still below normal and also when the latency for any response is already prolonged. When two stimuli of equal strength are applied repeatedly once a second at intervals even as far apart

as 200 to 300 milliseconds, the second response will frequently be greater than the first and have a shorter latent period. The above noted effects are regarded as an expression of alterations in the central excitatory state following activity. Especially noteworthy is the fact that even after an interval of a second, the effect of previous cortical activity persists, though the degree of effect is greatest when the stimuli are applied close together. Strychnine lengthens the interval between stimuli which will produce responses corresponding in size to the normal.

Effect of calcium on strychnine effects. When calcium is given intravenously after strychnine in the form of calcium gluconate, it eliminates

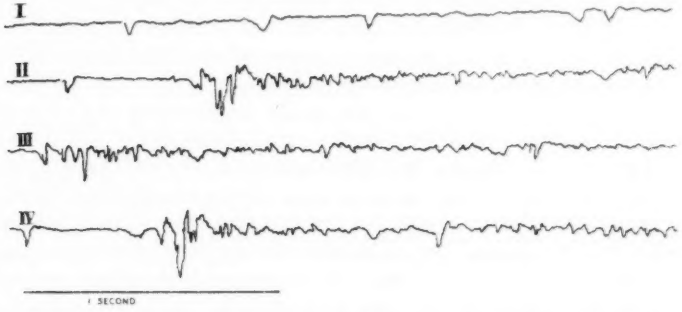

Fig. 6. Continuous record divided into four sections of cortical activity under strychnine to show the development of an increased excitatory state from slowly repeated saphenous nerve stimulation. Note that in line *I* only a small short first wave develops in response to stimulation. In line *II* such a response is followed by the later waves of the first response plus a spread of activity. In line *III* a full first response to saphenous nerve stimulation is followed immediately by a spread of activity. In line *IV* the first wave of the first response alone results and then after a second stimulation it appears, followed by the later waves of the first response plus spreading activity. The amplification has been reduced below where the ordinary beta waves show. It is felt that the first wave of the specific response to saphenous nerve stimulation results from activity in afferent cortical fibers. The later longer waves are considered to result in part at least from potentials developed in secondary neurons.

the prolongation of the period of latent addition. It likewise eliminates the large graded potentials elicited after peripheral nerve stimulation and also the large spontaneous potentials seen on the direct application of strychnine to the cortex. Local application of 10 per cent calcium gluconate to an area strychninized by local application results in the elimination of the strychnine effects so produced. It eliminates the facilitation effects referred to above in which a second stimulus of equal strength following a first is larger in area and has a shorter latency than the first. When a cat is given 1 to 2 grams of calcium gluconate intravenously, it can

tolerate three to four times the subcutaneous lethal dose of strychnine. Moderate strychnine convulsions can be stopped by intravenous calcium gluconate. Calcium has been found to have little effect on the threshold of nerve fibers until the concentration used is very great.

ANALYSIS OF RESULTS. The significance of the data secured by this research greatly depends upon the extent to which it is justifiable to project facts experimentally observable in certain parts of the nervous system into an explanation of the workings of other parts. More specifically, to what extent may one assume that strychnine modifications of axon, synapse, and neuron properties experimentally observable in the peripheral and visceral systems portray modifications which must also take place in the central nervous system? It is felt that our present knowledge not only does not preclude the belief that the same strychnine effects observed in peripheral and visceral synapses will also occur in the central nervous system, but strongly suggests it. Observable differences are regarded as due to differences in the levels of excitability of the structures involved. Fiber studies of the optic nerve (Bishop and Heinbecker, 1933) and the spinal cord (Gasser and Graham, 1933) indicate that their properties are similar to those of peripheral nerves and it may therefore be assumed that they would be similarly modified by strychnine. Synapse studies of the superior cervical ganglion by Eccles (1937) and of the oculomotor nucleus by Lorente de Nó (1935) indicate that here, too, observable properties, like the period of latent addition and the absolutely refractory period, are comparable. In nerve trunks and synapses differences in actual time values go hand in hand with differences in the time functions of the axons involved and are, therefore, to be expected. The synapses of the median nerve cord of the Limulus and the central nervous system are similar in the reaction to certain drugs, and histologically and functionally this cord may be considered a central nervous system in miniature. This being the case, what is found by further experiment, as well as what is already known, may be regarded as suggestive of the way the central nervous system reacts. In giving these examples, the inference is not to make the central nervous system and the median nerve cord of the Limulus identical in all their properties with the synapse neuron complex of the superior cervical ganglion in the cat and turtle. It is known, for example, that conduction through a peripheral synapse and synaptic influences on the autochthonous neurons or those which after excitation are associated with long potentials and with repetitive responses such as are found in the viscera and in the central nervous system are much different in their degree of susceptibility to nicotine depression. The *end-result* of synaptic activity is also not similar.

An analysis of the data concerning the influence of strychnine on

threshold throughout the nervous system leads to the conclusion that dilute concentrations lower it. In higher concentrations it always raises the threshold. The degree of lowering by a given concentration depends on the locus. The peripheral nerve, the peripheral synapse and the peripheral sympathetic neuron show the least effect. It is well marked in the Limulus nerve cord, the spinal cord, and the brain. It is in these loci that ganglion cells possessing autochthonous rhythmicity and ganglion cells which on excitation produce repetitive responses in their axons exist. It is believed that differences in the level of their threshold or excitatory state are responsible for the well established fact that strychnine affects primarily the central nervous system.

It has been shown that something similar to accommodation is likewise similarily modified throughout the nervous system by strychnine. The experimental results on the fiber are definite. Prolongation of the period of latent addition at the peripheral synapse-neuron junction, in the spinal cord and brain suggests a slowing of the process of accommodation. This permits the addition of excitatory influences over a longer time period with a resultant increase in the intensity and duration of the final response.

All parts of the nervous system are similarly affected by strychnine but different parts have different thresholds of absolute excitability and for augmentation by strychnine. It is noteworthy that the effect of strychnine on the median nerve cord of Limulus is to increase the area of its slow potentials and the repetitive nature of the axonal responses associated with such potentials. A similar result has been shown to follow its contact with the central nervous system. In the axon and in the outlying ganglia there is no increase in potential area and there is no tendency for repetitive responses. The inference is that the prolongation of the potentials and the tendency to repetitive responsiveness go hand in hand. Prolongation of the potential is regarded as an expression of diminished accommodation in the soma to intrinsic and extrinsic excitation. The somata concerned are those spontaneously rhythmical and those which on extrinsic stimulation are depolarized for a sufficient period to cause repetitive responses in the axon. The fact that calcium, which is known to increase the degree of accommodation in the axon (Hill, 1936), eliminates both the abnormal prolongation of the potentials and the increased repetitiveness is considered valid support for the conception that calcium opposes strychnine action by opposing its effects on accommodation. Other influences have been found to affect the area of the smooth potentials and the repetitive axon responses in Limulus. The inhibitory nerves to the heart shorten the potentials and slow the repetitive responses, the excitatory nerves lengthen them and increase the repetitive responses. Acetylcholine has an effect like the inhibitory nerve, epinephrine has an effect like the excitatory nerve. It

follows that in the intact animal under normal circumstances such substances could affect the response of nervous structures by an influence on their accommodation process.

The action of strychnine appears similar in some respects to that of anodal polarization. Anodal polarization (Blair, 1938) like strychnine opposes accommodation, it lengthens the period of latent addition and in weak concentrations it decreases recovery time. Strychnine lowers threshold. Anodal polarization elevates the electrical threshold but this does not necessarily truly reveal its effect on absolute threshold. Anodal polarization causes a definite increase in axon spike height and negative after-potential effects not produced by strychnine in the axon but strikingly so in the cell potentials of viscera and the central nervous system.

SUMMARY

Strychnine action on the nervous system appears to be brought about by changes in excitation and accommodation. In weaker concentrations threshold is lowered, in stronger concentrations it is raised. Accommodation is diminished. The period of latent addition is prolonged. Absolute refractoriness in the axon is increased.

The action of strychnine tends to be similar throughout the nervous system but the degree of change varies with the locus. Its effect is least in the axon, in the peripheral synapse and ganglion cell. It is greatest in the central nervous system and in certain visceral ganglia (Limulus polyphemus).

Strychnine in concentrations which result in tremendous potential increases in the Limulus heart, cord and the central nervous system of vertebrates depresses both the negative and positive after-potentials of the fiber.

The greatest action of strychnine is considered to be on the somata of the central nervous system, altering the duration and degree of their depolarization. The duration and degree of such depolarization determines the duration and frequency of the responses of the axon of the soma.

Increases in potential amplitude and duration produced by strychnine in the central nervous system go hand in hand with effects similar to those caused by a diminution of accommodation in the fiber. Calcium, which increases accommodation in the fiber, is an antagonist to such effects in the central nervous system. It eliminates the potential changes, consequently the changes in amplitude and duration of long potentials of the central nervous system are also regarded as due to changes effected by a diminution in accommodation.

The effects of strychnine simulate closely some of those of anodal polarization.

Strychnine action on the ganglion cells of the Limulus heart nerve is similar in many respects to that of extrinsic nerves excitatory to the heart and to that of epinephrine. Inhibitory nerves have an opposite effect and their action is similar to that of acetylcholine.

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PERSISTENT DIABETES FOLLOWING THE INJECTION OF ANTERIOR PITUITARY EXTRACT

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F. G. Young (1) has recently described the production in dogs of diabetes which continued for months after the cessation of intraperitoneal injections of increasingly larger doses of a carefully prepared extract of the anterior pituitary gland. Campbell and Best (2) have had a similar experience. In 1932 H. M. Evans (3) briefly reported the persistence of diabetes in 2 dogs after approximately 9 months' treatment with a growth hormone preparation. The present report confirms these workers and adds metabolic studies to illustrate the character of the diabetes so induced.

METHODS. A total of 6 male and 2 female dogs were given a saline extract of bovine anterior pituitaries made according to the technic described by Young (4).[1] The dosage is indicated by the number of grams of glands which were treated to produce the amount of extract given. For brevity this measurement of dosage will be designated as extract-grams. In general, the initial dose was 10 extract-grams which was continued for several days, then increased gradually to 35 extract-grams. All extract was administered intraperitoneally. The shaved abdomen was washed with soap and water and alcohol before each injection.

The diet consisted of weighed portions of varying amounts of the following: lean beef heart, sugar, and a "stock diet" composed of 55.7 per cent of ground meat, 40.7 per cent cracker meal, 1.5 per cent cabbage, and cod liver oil, iodized salt and bone ash 0.7 per cent of each. When the stock diet was not used, yeast, bone ash and cod liver oil were given. During the period of injections the animals were allowed to eat as much as they were able.

The dogs were weighed in the morning before feeding. They were kept in metabolism cages and daily urine collections were made, toluene being used as a preservative. Exercise was allowed for short periods after voiding. The total available carbohydrate of the diet was calculated in the usual manner using a D/N ratio of 3.65. The nitrogen content of the food represents an average of several determinations.

[1] The aid of Mr. J. O. Cole of Swift and Company in arranging for the collection of the glands is gratefully acknowledged.

The urine glucose was determined by the Benedict method, urine and food nitrogen by the micro-Kjeldahl, urine ketones by the Van Slyke method. Blood sugar was determined by a modification of the Folin micro method.

RESULTS. Three of the 8 dogs used became "permanently" diabetic. The course of the diabetes in such an animal is shown in figure 1. The glycosuria during the permanent phase following the withdrawal of anterior pituitary extract was of varying intensity in the 3 animals. Weight loss was fairly marked in the 2 more severe cases (nos. 2 and 3). These 2 dogs became distinctly worse with time as shown by increasing ketonuria and by the increasing proportion of the available carbohydrate which was

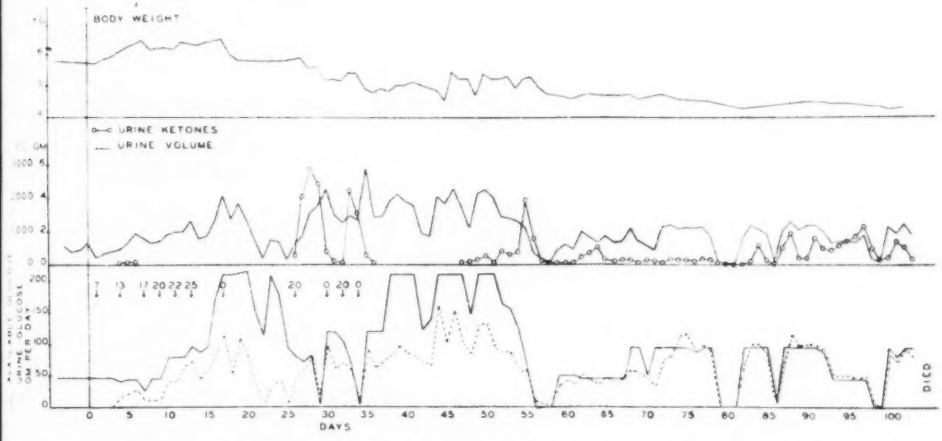

Fig. 1. Dog 2. The numbers over the arrows give the weight of anterior pituitary glands used in preparing the amount of extract injected daily until the subsequent arrow.

excreted. Dog 1 is still alive 6 months after the first injection of extract. Dog 2 died during a period of hot weather 71 days after stopping injections. The death of dog 3 on the 40th day after the cessation of injections was due to peritonitis subsequent to a biopsy of the liver and pancreas. The apparently normal strength, activity, and appetite of these animals was in contrast to that of the untreated depancreatized dog.

The period of injections before the onset of a permanent diabetes varied from 4 to 6 weeks. Extract from a total of 103, 72 and 81 grams of anterior pituitary per kilogram of original body weight was administered to dogs 1, 2 and 3 respectively.

Post injection studies: Glucose, nitrogen and acetone body excretion. The effects of fasting and of various diets are shown in table 1. It is quite

TABLE 1

Urinary glucose, nitrogen and ketone bodies in dogs after termination of injections

DOG NO.	PERIOD*	WT.	AVERAGE PER DAY: Food (B = Beef, S = Sucrose)	Urine glucose	Available carbohydrate†	Urine nitrogen	Food nitrogen	Urine ketones†	REMARKS
			Fasting						
	days	*kgm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mgm.*	
1	110–112 (67)	11.2	Fasting	0.5	0	4.0	0	0	
1	161–163 (118)	13.2	Fasting	0	0	2.6	0	0	
2	56–58 (23)	5.3	Fasting	5.0	0	3.2	0	670	Blood sugar at end of fast 215 mgm. %
2	79–81 (46)	4.6	Fasting	1.4	0	2.6	0	12	Blood sugar at end of fast 167 mgm. %
3	61–64 (31)	8.0	Fasting	4.7	0	3.0	0	8	Blood sugar at end of fast 313 mgm. %
			Fat diet						
1	94–100 (51)	10.3	Lard 100	0.5	10	3.4	0	0	
2	98–99 (65)	4.4	Lard 50	0.9	5	2.3	0	476	
			Meat diet						
1	69–72 (26)	11.2	B 1000	9.3	94	23.4	25.7	0	D/N = 0.39
1	73–76 (30)	11.2	B 1500	25.4	141	38.8	38.6	0	D/N = 0.66
1	138–140 (95)	12.7	B 1000	8.8	94	23.5	25.7	0	D/N = 0.37
2	66–68 (33)	4.7	B 500	44.0	47	12.5	12.9	279	D/N = 3.52
2	93–94 (60)	4.5	B 500	50.2	47	13.2	12.9	584	D/N = 3.80
3	45–49 (15)	8.7	B 1000	50.6	94	24.3	25.7	97	D/N = 2.08

* Days of periods are inclusive and are counted from the first injection of pituitary extract. Number of days from last injection is in parenthesis.

† See text.

TABLE 1—*Concluded*

DOG NO.	PERIOD*	WT.	AVERAGE PER DAY: Food B = Beef S = Sucrose	Urine glucose	Available carbohydrate†	Urine nitrogen	Food nitrogen	Urine ketones†	REMARKS
			Meat and sucrose diet						
	days	*kgm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*mgm.*	
1	65–68 (22)	11.1	B 1000, S 100	53.7	194	25.5	25.7	0	
	88–91 (45)	12.2	B 1000, S 100	40.7	194	24.7	25.7	0	
	121–122 (79)	13.0	B 1000, S 100	90.2	194	26.5	25.7	0	Diabetes more severe
2	73–75 (40)	4.7	B 500, S 50	102.6	97	13.1	12.9	361	
	90–92 (57)	4.5	B 500, S 50	93.8	97	13.8	12.9	574	
	102–103 (69)	4.3	B 500, S 50	87.1	97	10.8	12.9	420	Very thin
3	42–44 (12)	8.5	B 1000, S 100	124.8	194	24.8	25.7	0	
	65–67 (35)	7.5	B 1000, S 100	160.7	194		25.7	1030	Diabetes more severe

evident that none of these animals displayed the metabolic phenomena characteristic of the fasting, completely depancreatized dog, although the metabolic behavior of dog 2 on a meat or meat and sucrose diet seems similar to that of such an animal. The high D/N ratio is of interest. The glucose excretion at first was less than the amount available in the diets. This condition changed so that (dogs 2 and 3) the glucose output equalled the intake. In spite of this it will be noted that fasting and fat feeding reduced the glucose and nitrogen excretion to levels well below those of the fasting, depancreatized animal.

The nitrogen excretion during fasting is within the range of the normal animal. When meat is eaten dogs 1 and 3 appear to be in nitrogen equilibrium. For the fasting periods the urinary nitrogen has been calculated per kilogram and per square meter, and the results compared with those of normal and depancreatized animals. The nitrogen output of dog 2 comes within the range of the depancreatized animals on the body weight but not on the square meter basis. Dogs 1 and 3 are clearly within the

normal range. This agrees with the variations, already noted, in the severity of the diabetes of the injected animals.

The urinary ketones were also greatly decreased during the fasting and fat feeding periods. Thus dog 2 (fig. 1) during the fasting period including the 56th, 57th and 58th days excreted 1529 mgm., 305 mgm. and 113 mgm. on the days indicated. This is to be compared with an average of 1792 mgm. per day for the previous 3 days while eating meat. During the 98th and 99th days while receiving a fat diet he excreted 553 and 392 mgm. of ketone bodies. During the previous 3 days, while eating 500 grams of beef heart per day, the daily ketone body excretion was 700 mgm., 875 mgm., and 1160 mgm. A similar effect was noted during the fasting period of dog 3. Dog 1 had no ketonuria at any time during the post injection phase.

TABLE 2

Glucose tolerance tests (1.8 gram per kgm. by mouth)

DOG NO.	TIME AFTER LAST INJECTION	BLOOD SUGAR				
		Before	½ hr.	1 hr.	2 hrs.	3 hrs.
A. Normal – 5 tests						
	days	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Average		79	115	114	103	80
Range		77–81	93–146	97–134	89–114	66–96
B. Diabetic						
1	48	138	219	271	282	
	58	89	196	259	222	177
	66	173	343	394	267	227
2	25	215	257	299	335	
	47	297	434	453	390	303
	69	239	384	370	361	338
3	13	253	299	337	337	

Blood sugar studies. Sample fasting blood sugars and the results of oral glucose tolerance tests are shown in table 2. As would be expected, the curves were of a diabetic type. They, like the glucose excretion, demonstrate the imparied utilization of carbohydrate. For at least 3 days prior to the tests the normal animals were fed on meat or stock diet. The test on dog 1 on the 58th day followed 7 days on a diet of 100 grams lard daily. The low fasting blood sugar was not associated with any striking change in the response to glucose.

Following the ingestion of meat there was a rise of blood sugar to 229

mgm. per cent in dog 1 and to 360 mgm. per cent in dog 2 from fasting levels of 134 mgm. per cent and 274 mgm. per cent respectively.

Response to insulin. Insulin tests were performed in dog 1 (table 3). These are compared with the response to insulin in normal animals and are in contrast to the lack of response to insulin in dogs *during* the period of extract treatment. The results during injection are in agreement with previous studies (5) (6). The persistence of a diabetes which will respond readily to insulin is in accord with other evidence for the ultimate pancreatic nature of this type of diabetes.

TABLE 3

Response to insulin (0.1 unit per kgm. intravenously)

DOG NO.	TIME OF OR AFTER INJECTION	BLOOD SUGAR				
		Before	Minutes			
			15	30	45	60
A. Normal—5 tests						
	days	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Average		80	52	60	62	71
Range		74–85	45–57	55–64	60–64	61–78
B. During pituitary extract injections						
10	26	177	184	181	189	189
9	5	229	250	242	255	248
C. After pituitary extract injections						
1	117	204	116	74	103	106
1	132	161*	83	56	66	82
1	164	114†	66	56	70	69

* After 12 days' diet of 75 grams lard and 200 grams beef.
† After 7 days' fasting.

Autopsies. A liver biopsy obtained from dog 3 on the 69th day, after a 24 hour fast, contained 2.96 per cent glycogen. At autopsy following its death from peritonitis two days later the liver fatty acids were 6.85 per cent. The liver fatty acids of dog 2 which died during hot weather were found to be 9.52 per cent.

The biopsy from the tail of the pancreas of dog 3 showed extremely few islands of Langerhans; in fact, they were only found by careful searching. These islands were small and the cells shrunken with pyknotic nuclei. Hyaline and fibrous scars were not seen. The acinar cells were well preserved.

The sections of the pancreas of dog 2 showed islands which were small

and few in number. Detailed study was prevented by post mortem changes.

Discussion. Our experience in the production of a permanent diabetes by increasing doses of anterior pituitary extract is similar to that of Young (1) and of Campbell and Best (2). The effect of a fat diet in greatly reducing glycosuria and ketonuria has been briefly noted by Marks and Young (7) in one of their animals.

It appears from the reports of Richardson and Young (8), and Campbell and Best (2), as well as from preliminary studies of our own pathological material, that the islands of Langerhans undergo varying degrees of damage. The two dogs reported by Richardson and Young (8) showed in one case almost all island tissue to be hyalinized, and in the other varying grades of cytoplasmic granule depletion. The pancreas of the dog described by Campbell and Best (2) showed an extreme degree of hydropic degeneration of the islands of Langerhans and the insulin content of the pancreas was less than 2 units. Houssay and Foglia (9) have reported evidence of decreased insulin secretion in the pancreas of dogs injected with anterior pituitary extract.

The concept that this post-injection diabetes is due to a decrease in the functional capacity of the islands of Langerhans is also supported by the course of the diabetes and the metabolic studies. The association of a high D/N ratio when on a meat diet with the extremely low glycosuria during fasting and fat feeding as found in dog 2 has its counterpart in the partially depancreatized dogs fully described by Langfeldt (10). The unimpaired insulin sensitivity in dog 1 suggests that the anterior pituitary glycotropic hormone mechanism is not abnormally active after injections are stopped. Campbell and Best (2) considered their dog to have insulin requirements of a depancreatized dog on a similar diet. However, one of Young's dogs required a high insulin dosage on a relatively great food intake. Our dogs did not receive insulin other than for the tests indicated.

SUMMARY

1. Persistent diabetes was produced in three of eight dogs by progressively larger doses of anterior pituitary extract. The results in general are similar to those of Young and of Campbell and Best.

2. While the intensity of the diabetes varied, the metabolic behavior of the animals was similar to that of partially depancreatized dogs.

3. Histological evidence also indicates damage to the islands of Langerhans.

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THE DEFATIGUING EFFECT OF ADRENALINE

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The literature concerning the effect of adrenaline on muscular fatigue is contradictory. Cannon and Nice (1913) and Gruber (1913) reported a defatiguing action in normal muscle stimulated through its nerves. Mertens, Rein and Valdecasas (1936) were unable to observe any effect of adrenaline on the tension of fatigued muscle thus stimulated.

A similarly confusing situation exists with regard to the action of adrenaline on the fatigue of denervated muscles stimulated directly. While Gruber (1914) found a defatiguing effect in these circumstances, Guglielmetti (1922) and Corkill and Tiegs (1933) claim that adrenaline defatigues only at the neuromuscular junction, because its action is seen only in muscles indirectly stimulated.

The purpose of the present work was to explain these contradictory results by the study, under different experimental conditions, of the effect of adrenaline on the fatigue of normal and denervated muscles. In the discussion an attempt is made to account for the results obtained in terms of the concept of chemical mediation of the motor nerve impulses.

METHODS. Cats were used, usually under dial anesthesia (Ciba, 0.7 to 0.8 cc. per kgm. intraperitoneally). In some cases a spinal preparation was made during brief etherization. When necessary a cannula was inserted into the trachea for artificial respiration. The muscles studied were the gastrocnemius-soleus, the quadriceps and the posterior auricular. In some experiments they were denervated by aseptic section of a sciatic, femoral or facial nerve, respectively, 5 to 30 days before the experiments. When the muscles of the leg were employed it was fixed by means of drills in the tibia or femur. For study of the facial muscle the head was held by a Czermak clamp.

The contractions of the muscles were recorded on a kymograph by attaching the tendon to the short arm of a writing lever pulling against a rubber band. The magnification was 6- to 10-fold.

For indirect stimulation the electrodes were shielded silver wires. When the muscles were stimulated directly, steel needles were inserted into their bodies and tendons.

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The stimuli used varied with the purpose of the experiment. Occasionally a Harvard induction coil with 5 volts in the primary circuit was employed. Repetitive stimulation at different frequencies was obtained from a multivibrator circuit. Condenser discharges of various capacities were also used.

The injections were usually made into a jugular vein. In some cases, in which the quadriceps muscle was used, injections were made into the femoral artery a few millimeters above the branch to the muscle.

The adrenals were routinely ligated in the experiments in which acetylcholine was injected; atropine (1 mgm. per kgm.) was given in these experiments and in those in which prostigmin was employed. Usually 2 to 3 mgm. of ergotoxine (Burroughs, Wellcome) per kgm. were given. Prostigmin (Roche), when used, was injected in doses of 0.5 mgm. per kgm. The adrenaline was the adrenalin of the Parke, Davis Company.

In several experiments the blood pressure was recorded by a mercury manometer connected with the carotid artery.

RESULTS. A. *Normal muscles.* Injections of adrenaline during repetitive stimulation of motor nerves produced different effects on muscular fatigue, dependent on the doses employed, the frequency of stimulation and the presence or absence of ergotoxine.

When the frequency of stimulation was lower than 20 per minute adrenaline in any dose had no effect.

Small doses (from 10 to 25 γ) injected intravenously usually led to a defatiguing effect if the frequency of stimulation was 20 to 120 per minute. At higher frequencies, as a rule, these small doses had a depressant effect on the muscular contraction (fig. 1*a*).

Large doses of the hormone (100 γ) had a depressant effect at any frequency higher than 20 per minute.

If adrenaline was administered after ergotoxine the depressant effect did not appear at any dose and the defatiguing effect was stronger than before the administration (fig. 1*b*).

After injections of prostigmin indirect stimulation of muscles with a frequency of about 100 per sec. results in characteristically complex changes of tension (cf. Rosenblueth and Morison, 1937). An early fall of the tension is followed by a rise and then a final slow fall which represents the fatigue curve. Adrenaline caused a depression when injected during the second rise of tension (fig. 2) and had a defatiguing effect when injected during the terminal fall. The depression in the second rise does not result from vasoconstriction, for it occurs after ergotoxine (see fig. 2).

B. *Denervated muscles stimulated electrically.* The observations reported by v. Euler and Gaddum (1931) and Bülbring and Burn (1936), that adrenaline often produces a slow contractile response of denervated muscle, were confirmed. Also, the observations made on denervated muscles by

Gruber (1914) were confirmed. In all cases a defatiguing effect of adrenaline administered intravenously or intra-arterially was present, regardless of the change observed in the blood pressure, the frequency of stimulation used and the doses of the hormone injected. After ergotoxine the defatiguing action was greater.

The influence of adrenaline on the electrical excitability of denervated muscles was studied in several animals by changing the duration of the shocks employed. With different lengths of shocks no significant difference was observed in the improvement of muscular contraction produced by adrenaline during fatigue. In other animals strength-duration curves of 6 different capacities (from 0.1 to 5 μ farads) were constructed from observations made before and immediately after injections of adrenaline

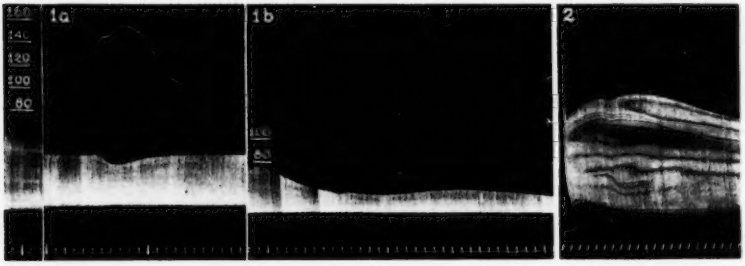

Fig. 1. Adrenaline in normal fatigued muscle. Lower record: gastrocnemius-soleus stimulated indirectly throughout at the rate of 180 per minute. Upper record: blood pressure. Upper signal: intravenous injection of 25γ of adrenaline. Lower signal: 5-second intervals. The record preceding 1*a* shows the length of the contraction before the onset of fatigue. 1*a*. Before ergotoxine. 1*b*. After ergotoxine (2 mgm. per kgm.).

Fig. 2. Depression in non-fatigued normal muscle after ergotoxine and prostigmin. Quadriceps stimulated indirectly at the rate of 180 per minute. Upper signal: intra-arterial injection of 20γ of adrenaline. Lower signal: 5-second intervals.

in doses sufficient to defatigue (20 to 100 γ). In these animals adrenaline caused no significant change of the strength-duration curve of the denervated gastrocnemius and quadriceps.

C. *Denervated muscles stimulated by acetylcholine.* A depressant effect of adrenaline on the response of denervated muscle to acetylcholine was reported by Frank, Nothmann and Hirsch-Kauffmann (1922) and by Gasser and Dale (1926). Further observations of Dale and Gaddum (1930) supported this conclusion. Brown (1937), on the other hand, found that the response of normal muscle to acetylcholine was not significantly affected by adrenaline.

In 21 cats the influence of adrenaline on the response of denervated muscles to acetylcholine was studied under different experimental condi-

tions. Seven of the animals were made spinal under ether and the rest were anesthetized with dial. Adrenaline was always injected intravenously and acetylcholine either intravenously or intra-arterially. The doses of adrenaline were from 20 to 200 γ, and the doses of acetylcholine from 5 γ to 2 mgm. The gastrocnemius, the quadriceps and the posterior auricular were employed 5 to 30 days after denervation. In one experiment a normal extrinsic eye muscle was used.

In the experiments in which ergotoxine was not injected it was found that the responses of the muscles to acetylcholine were decreased and then increased by adrenaline. The depression of the responses occurred simultaneously with the rise of blood pressure produced by the hormone (fig. 3). In the observations made after ergotoxine the blood pressure was decreased or not importantly modified by adrenaline. The responses to acetylcholine were then only increased by adrenaline (fig. 4).

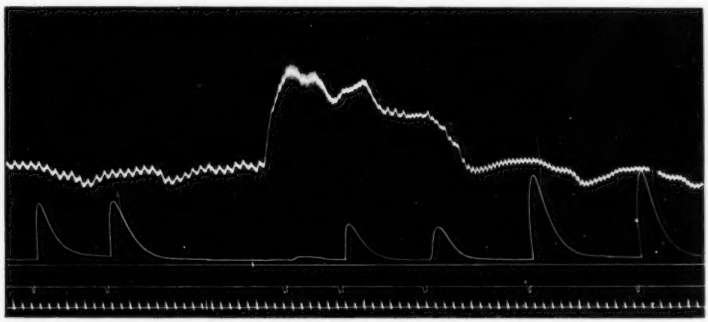

Fig. 3. The influence of adrenaline in the response of denervated muscle to acetylcholine. Upper record: blood pressure. Lower record: quadriceps denervated for 7 days. Dial; atropine (1 mgm. per kgm.); adrenals ligated. At upper signal 100γ adrenaline intravenously. At middle signals 10γ acetylcholine intra-arterially. Time: 5-second intervals.

It has been shown (Rosenblueth and Luco, 1937) that acetylcholine elicits mainly or exclusively contractions in a freshly (5 or 6 days) denervated muscle. When the muscle has been denervated longer (15 to 30 days) contractures predominate in the response to acetylcholine. Since the results reported above were observed in muscles denervated from 5 to 30 days, it may be concluded that both the contraction and the contracture are modified by adrenaline (fig. 4).

After injections of ergotoxine much larger doses of acetylcholine than before were required to induce the same muscular response. Usually it was necessary to wait about 10 minutes after injecting ergotoxine to observe consistent responses to acetylcholine. The different results reported by Gasser and Dale (1926) may have been due to injections of

acetylcholine and adrenaline being made shortly after ergotoxine was given.

In the present observations there was no difference in the results obtained in cats under dial and in the spinal preparations. Similarly the intravenous and the intra-arterial injections of acetylcholine had the same effects.

D. *Sympathetic stimulation and response of denervated muscles to acetylcholine.* In some animals the facial nerve was cut and after the usual interval for degeneration the response of the posterior auricular muscle to acetylcholine was tested before, during and after stimulation of the cervical sympathetic trunk.

Fig. 4. The influence of adrenaline on the response of denervated muscle to acetylcholine after ergotoxine (3 mgm. per kgm.). Gastrocnemius denervated for 8 days. Spinal preparation; adrenals ligated; atropine (1 mgm. per kgm.). At upper signal 200γ adrenaline intravenously. At middle signals 100γ acetylcholine intravenously. Notice that the contraction and the contracture are increased after adrenaline.

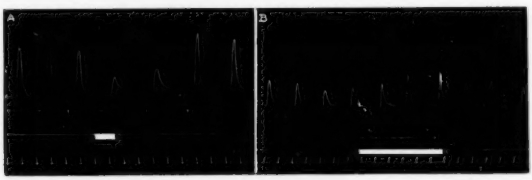

Fig. 5. Sympathetic stimulation and responses to acetylcholine of denervated muscles. Posterior auricular muscle denervated for 10 days. Atropine (1 mgm. per kgm.). Upper signal: intravenous injections of acetylcholine; middle signal: stimulation of the cervical sympathetic trunk. Lower signal: 30-second intervals. A. Before ergotoxine. B. After ergotoxine.

In some of the experiments without ergotoxine the results reported by Dale and Gaddum (1930) were confirmed, i.e., the muscular response was completely suppressed for a short period by sympathetic stimulation. In other similar experiments the contractions due to acetylcholine were only decreased during the stimulation (fig. 5A). In one experiment, however, excitation of the sympathetic was accompanied by increased responses to repeated uniform doses of acetylcholine.

When ergotoxine was previously administered the contractions of the posterior auricular muscle resulting from acetylcholine were increased

during sympathetic stimulation (fig. 5B). This increase was greater in the experiments in which the superior cervical sympathetic ganglion had been previously denervated and the postganglionic fibers were stimulated.

Discussion. As regards the contradictory reports mentioned in the introduction, the present results confirm the observations of Cannon and Nice in normal muscle (section A) and Gruber in denervated muscle (section B). The failure of Mertens, Rein and Valdecasas to observe the defatiguing effect of adrenaline in normal muscle stimulated through the nerve may be due to the small dose of hormone (5 γ) they used. The results reported by Corkill and Tiegs on denervated muscles may be explained by their use of frogs, whereas Gruber's study and the present observations were made on mammalian muscles. It is not possible to explain the results obtained by Guglielmetti, because he does not specify the dose of adrenaline he used nor the conditions of his experiments.

The possibility that the defatiguing effect of adrenaline on muscular contraction might be due to its action on the blood vessels has been discussed by earlier workers. Cannon (1929) has summarized the evidence which supports the conclusion that the effect is not exclusively due to blood-pressure changes, but is specific.

The action of adrenaline on blood vessels in active muscles is still in question (see Burn, 1938). The results reported in sections A and C confirm indirectly the conclusion of Bülbring and Burn (see Burn, 1938) that adrenaline causes vasoconstriction in active muscles. The depression observed with large doses in muscles indirectly stimulated (section A; fig. 1) occurs at the height of the rise of the blood pressure. This depression may be explained as due to a constriction of the vessels of the muscle, since it disappears after ergotoxine (fig. 1*b*). The same argument may be used to account for the diminished response of denervated muscles to acetylcholine (section C; fig. 3) after injections of adrenaline. Similarly, the reduced response to acetylcholine during sympathetic stimulation (section D; fig. 5) may be due to local vasoconstriction, since it disappears after ergotoxine.

The observation that ergotoxine decreases responses of muscle to acetylcholine (section C) contrasts with the well-known inhibitory action of the drug on cholinesterase. It may be concluded that ergotoxine has some influence upon the muscle by which the threshold to acetylcholine is increased.

The effects of sympathetic stimulation on the response of denervated muscle to acetylcholine closely resemble those of adrenaline. The depressant effect observed before ergotoxine has been already explained as due to vasoconstriction. The increase of the responses to acetylcholine after ergotoxine during sympathetic stimulation may be due to a decrease of the threshold to acetylcholine caused by the sympathin liberated locally.

This increase was greater when the ganglion was previously denervated, probably because, as Luco and Lissák (1938) showed, the amount of sympathin released in this condition is greater than normal. The results reported in section C support the inference that adrenaline lowers the threshold to acetylcholine.

As stated in sections A and B adrenaline defatigues normal muscles stimulated indirectly and denervated muscles stimulated directly. In the first case, as is well known, fatigue occurs at the neuromuscular synapses. Adrenaline in this case should therefore act through the neuromuscular junction. But in the denervated muscle stimulated directly, where the neuromuscular synapses do not exist, adrenaline should act directly on the muscle.

The bearing of the results on the several theories advanced to explain the transmission of motor nerve impulses to skeletal muscle may now be discussed.

If the muscles were stimulated by the action-potential of the nerve, the defatiguing influence of adrenaline in normal muscles indirectly stimulated might be explained as due to either a change in the action-potential of the nerve or to an increase of the electrical excitability of the muscle. There is no evidence that adrenaline modifies nerve action-potentials. As reported in section B the electrical excitability of muscle does not change after administration of adrenaline. The defatiguing action of the hormone on muscles indirectly stimulated remains, therefore, unexplained in terms of the theory of electrical transmission. On the other hand, this defatiguing action is readily explained if acetylcholine is the chemical mediator of the motor nerve impulses (Brown, Dale and Feldberg, 1936). In terms of the chemical theory, the fatigue of the neuromuscular junction denotes a decrease to subthreshold levels of the quanta of acetylcholine liberated by the motor nerve impulses.

Adrenaline, by lowering the threshold to acetylcholine (section C; figs. 3 and 4), would permit these decreased amounts, liberated during fatigue, to become effective.

The arguments used above in discussing the defatiguing effect of adrenaline may be used to explain partly the Orbeli effect (see Orbeli, 1924). The phenomenon consists in recovery of strength of contraction in fatigued skeletal muscles when sympathetic nerves are stimulated. The sympathin liberated during sympathetic stimulation should have effects similar to those of adrenaline, i.e., a lowering of the threshold to acetylcholine (section D; fig. 5).

A further support to the theory of humoral activation of skeletal muscles is given by the experiments on normal muscle stimulated indirectly after the administration of prostigmin (section A; fig. 2). The complex curves of tension observed in these experimental conditions are readily accounted

for on the basis of the chemical theory (Rosenblueth and Morison, 1937). The early fall of tension could be due to an accumulation of acetylcholine above the paralytic threshold; the subsequent rise would be due to the decline of the quanta so that the concentration of acetylcholine decreases into the effective range; the final slow fall of tension is explicable as the consequence of a continuing decline of the quanta into the subthreshold zone. If this explanation is accepted and if adrenaline lowers the threshold to acetylcholine, the injections of the hormone during the second rise should produce a depression in the muscular tension. This suggestion was confirmed by the experimental results reported in section A.

SUMMARY

The defatiguing action of adrenaline was studied in cats, on normal muscles stimulated indirectly and on denervated muscles stimulated directly. Instead of this defatiguing effect a depression sometimes occurred when injections were made without ergotoxine (fig. 1*a*). After ergotoxine no depressions were encountered (fig. 1*b*; sections A and B). The depression without ergotoxine is interpreted as due to vasoconstriction (p. 201). In non-fatigued normal muscles adrenaline may, however, produce a decrease of tension when administered after prostigmin. This effect is not vascular because it appears after ergotoxine (fig. 2).

The doses of adrenaline employed do not modify the electrical excitability of denervated muscles.

Adrenaline increases the responses of denervated muscles to acetylcholine (section C). Ergotoxine favors this action (figs. 3 and 4).

Stimulation of the cervical sympathetic increases the responses of the facial muscles to acetylcholine after section and degeneration of the facial nerve (fig. 5).

The results are discussed from the standpoints of the mode of action of adrenaline and the mode of transmission of the motor nerve impulses.

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